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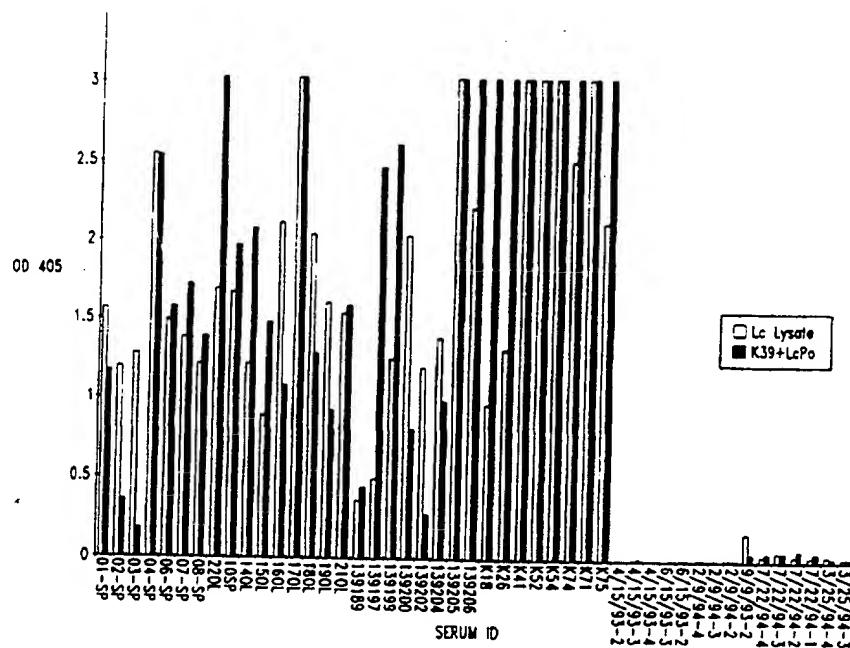


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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(30) Priority Data: 08/428,414	21 April 1995 (21.04.95)	US	
(71) Applicant: CORIXA CORPORATION [US/US]; Suite 464, 1124 Columbia Street, Seattle, WA 98104 (US).			(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(72) Inventor: REED, Steven, G.; 2843-122nd Place N.E., Bellevue, WA 98005 (US).		Published	<i>Without international search report and to be republished upon receipt of that report.</i>
(74) Agents: MAKI, David, J. et al.; Seed and Berry L.L.P., 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104- 7092 (US).			

(54) Title: COMPOUNDS AND METHODS FOR DIAGNOSIS OF LEISHMANIASIS



(57) Abstract

Compounds and methods are provided for diagnosing *Leishmania* infection. Disclosed compounds include polypeptides that contain at least an epitope of the *Leishmania chagasi* acidic ribosomal antigen LcP0, or a variant thereof. Such compounds are useful in a variety of immunoassays for detecting *Leishmania* infection and for identifying individuals with asymptomatic infections that are likely to progress to acute visceral leishmaniasis. The polypeptide compounds are further useful in vaccines and pharmaceutical compositions for preventing leishmaniasis.

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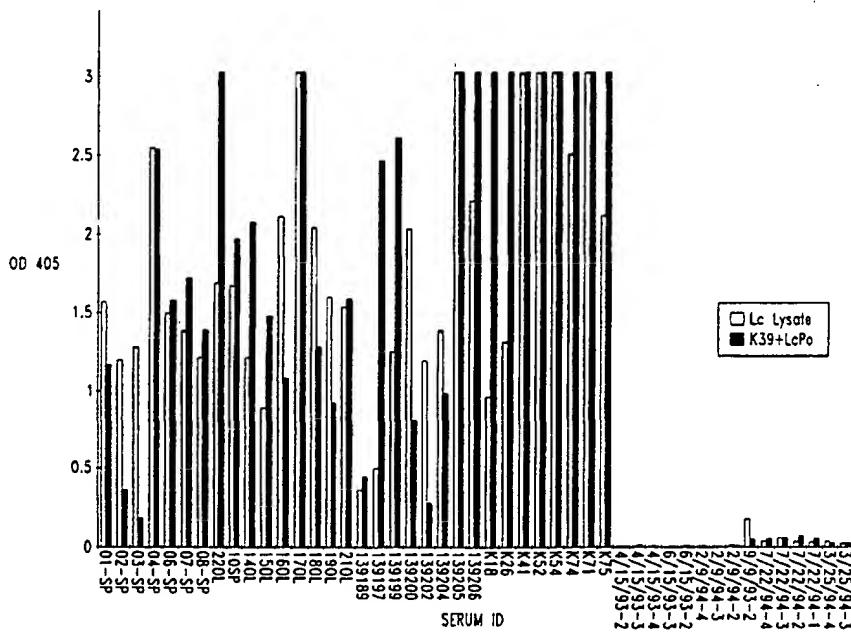
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(71) Applicant: CORIXA CORPORATION [US/US]; Suite 464, 1124 Columbia Street, Seattle, WA 98104 (US).			
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(54) Title: COMPOUNDS AND METHODS FOR DIAGNOSIS OF LEISHMANIASIS



(57) Abstract

Compounds and methods are provided for diagnosing *Leishmania* infection. Disclosed compounds include polypeptides that contain at least an epitope of the *Leishmania chagasi* acidic ribosomal antigen LcPO, or a variant thereof. Such compounds are useful in a variety of immunoassays for detecting *Leishmania* infection and for identifying individuals with asymptomatic infections that are likely to progress to acute visceral leishmaniasis. The polypeptide compounds are further useful in vaccines and pharmaceutical compositions for preventing leishmaniasis.

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INTERNATIONAL SEARCH REPORT

International Application No
PLI/US 96/05472

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/569 C07K14/44 C12N15/12 C12N15/85 C12N1/21
A61K39/005 G01N33/543 A61K39/002 A61K39/008

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>INFECTON AND IMMUNITY, vol. 62, no. 5, 1 May 1994, CHICAGO IL USA, pages 1643-1651, XP000604822 Y.A.W. SKEIKY ET AL.: "Antigens shared by Leishmania species and Trypanosoma cruzi: immunological comparison of the acidic ribosomal P0 proteins." see the whole document</p> <p>---</p> <p style="text-align: center;">-/-</p>	1,3-5, 26,33,36

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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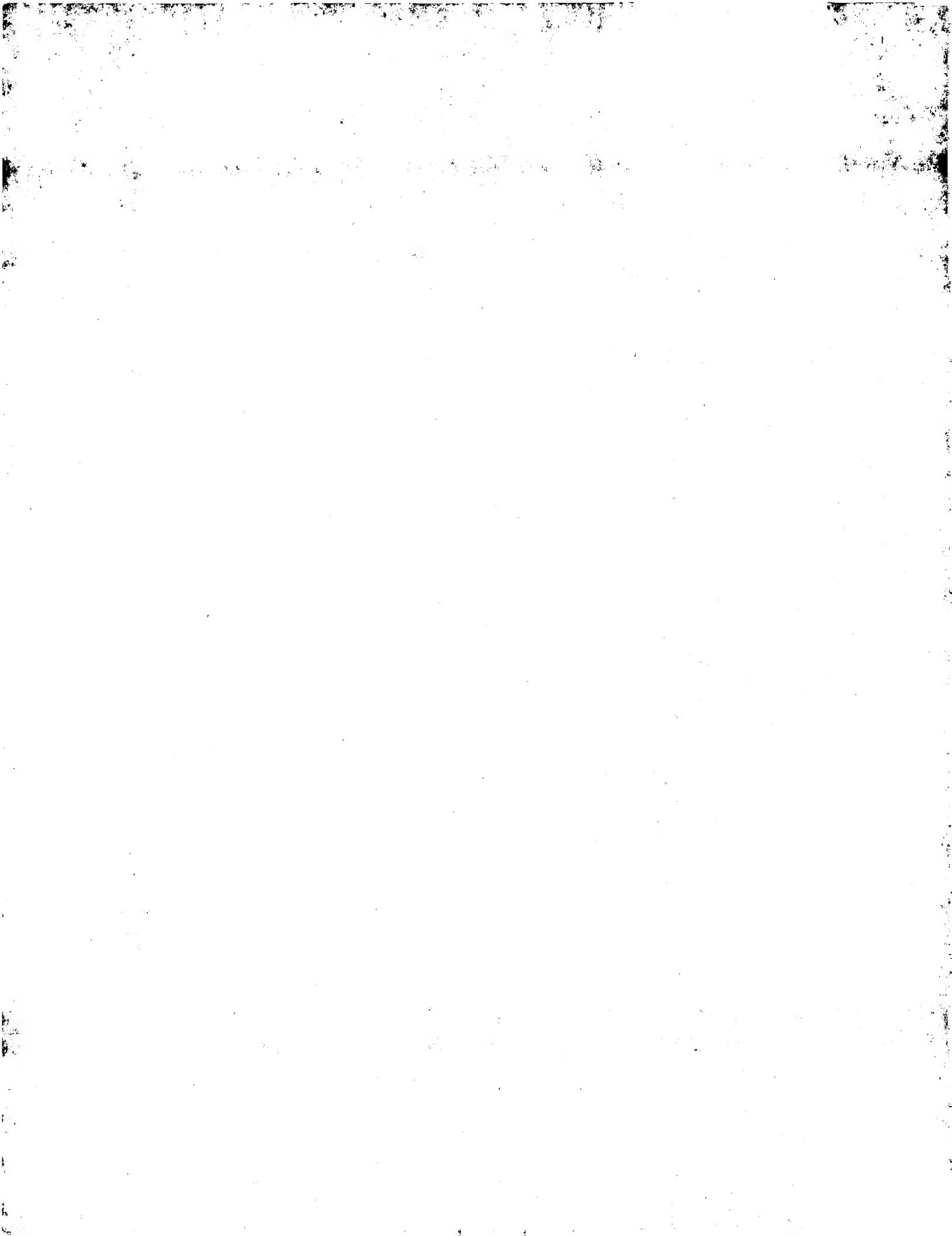
C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF EXPERIMENTAL MEDICINE, vol. 176, no. 1, 1992, NEW YORK NY USA, pages 201-211, XP000603651 Y.A.W. SKEIKY ET AL.: "Cloning and expression of Trypanosoma cruzi ribosomal protein P0 and epitope analysis of anti-P0 autoantibodies in Chagas' disease patients." see the whole document ---	1-42
A	US,A,5 304 371 (S.G. REED) 19 April 1994 cited in the application see the whole document ---	1-42
A	WO,A,93 16199 (S.G. REED) 19 August 1993 see the whole document -----	1-42

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/05472

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5304371	19-04-94	CA-A-	2129747	15-08-93
		EP-A-	0649475	26-04-95
		WO-A-	9316199	19-08-93
		US-A-	5413912	09-05-95
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WO-A-9316199	19-08-93	US-A-	5304371	19-04-94
		CA-A-	2129747	15-08-93
		EP-A-	0649475	26-04-95
		US-A-	5413912	09-05-95
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Description**COMPOUNDS AND METHODS FOR DIAGNOSIS OF LEISHMANIASIS**Technical Field

The present invention relates generally to the serodiagnosis of *Leishmania* infection. The invention is more particularly directed to the use of one or more *Leishmania* polypeptides in methods and diagnostic kits to screen individuals and blood supplies for *Leishmania*, and to identify those asymptomatic individuals that are likely to progress to acute visceral leishmaniasis. The invention is also directed to vaccines and pharmaceutical compositions for immunizing an individual against leishmaniasis.

Background of the Invention

Leishmania organisms are intracellular protozoan parasites of macrophages that cause a wide range of clinical diseases in humans and other animals. In some infections, the parasite may lie dormant, and an infected host may be asymptomatic for many years. In other cases, particularly in immunocompromised individuals, the host may develop one of a variety of forms of leishmaniasis. This disease may be subclinical visceral leishmaniasis (VL) or asymptomatic in nature. Patients with subclinical or asymptomatic disease usually have low antibody titers which fall into the gray zone in immunological tests using whole parasites or parasite lysates. Isolation of parasites from these patients is also extremely difficult. Subclinical patients will in some cases progress to acute disease, but often will self-heal. They exhibit mild symptoms of malaise, diarrhea and intermittent hepatomegaly. Asymptomatic patients, in addition to low antibody titers, also display strong, positive delayed hypersensitivity to leishmanial antigens. Alternatively, leishmaniasis may be manifested as a cutaneous disease, which is a severe medical problem but is generally self-limiting, or as a highly destructive mucosal disease. Finally, and most seriously, the disease may be manifested as an acute visceral infection involving the spleen, liver, and lymph nodes, which is generally a fatal disease. Symptoms of acute visceral leishmaniasis include hepatosplenomegaly, fever, leukopenia, anemia and hypergammaglobulinemia.

Leishmaniasis is a serious problem in much of the world, including Brazil, China, East Africa, India and areas of the Middle East. The disease is also endemic in the Mediterranean region, including southern France, Italy, Greece, Spain, Portugal and North Africa. The number of cases of leishmaniasis has increased dramatically in the last 20 years, and millions of cases of this disease now exist worldwide. About 2 million new cases are diagnosed each year, 25% of which are visceral leishmaniasis (VL).

There are 20 species of *Leishmania* that infect humans. Of these species, VL is generally caused by *L. donovani* in Africa, China, the Middle East and India, *L. infantum* in southern Europe and North Africa, or *L. chagasi* in Latin America. In general, *Leishmania* species are transmitted to humans and other mammals, primarily the dog, by the bite of a phlebotomine sand fly.

Early diagnosis of leishmaniasis is crucial for successful treatment, but is difficult to achieve with existing techniques. There are no distinctive signs or symptoms of the disease. Parasite detection methods have been used, but such methods are not sensitive or practical. Current serological tests (using, for example, ELISA or immunofluorescence techniques) typically use whole or lysed parasites, and are generally insensitive and prone to cross-reaction with a variety of other diseases. Such methods often fail to detect the potentially fatal disease early enough to allow effective treatment, since they rely on the detection of antibodies that are present during the acute phase of the disease.

Accordingly, there is a need in the art for more sensitive and specific methods for detecting *Leishmania* infection, and for identifying those asymptomatic *Leishmania* infections that are likely to progress to acute visceral infections. The present invention fulfills these needs and further provides other related advantages.

Summary of the Invention

Briefly stated, this invention provides compounds and methods for detecting and protecting against leishmaniasis in individuals and in blood supplies. In one aspect, the present invention provides methods for detecting asymptomatic or sub-clinical *Leishmania* infection in a biological sample, comprising: (a) contacting a biological sample with a polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; and (b) detecting in the biological sample the presence of antibodies that bind to the polypeptide, thereby detecting asymptomatic or sub-clinical *Leishmania* infection in the biological sample.

In a related aspect, the present invention provides methods for detecting *Leishmania* infection in a biological sample, comprising: (a) contacting a biological sample with a first amino acid sequence comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; (b) contacting the biological sample with a second amino acid sequence comprising Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and (c) detecting in the

sample the presence of antibodies that bind to one or both of the amino acid sequences, thereby detecting *Leishmania* infection in the biological sample.

In yet another related aspect of this invention, methods are provided for identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis. In one embodiment, the method comprises: (a) contacting a biological sample obtained from a patient afflicted with asymptomatic or subclinical leishmaniasis with a polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and (b) detecting in the sample the presence of antibodies that bind to the polypeptide, thereby identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis.

In another embodiment, the method comprises: (a) contacting a biological sample obtained from a patient afflicted with asymptomatic or subclinical leishmaniasis with a first polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; (b) independently contacting the biological sample with a second polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and (c) detecting in the sample the presence of antibodies that bind to the first and/or second polypeptides, thereby identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis.

In another aspect of this invention, polypeptides are provided comprising amino acids 306-322 of SEQ ID NO:2.

Within related aspects, diagnostic kits for diagnosing leishmaniasis are provided. In one embodiment, this invention provides kits for detecting asymptomatic or sub-clinical leishmaniasis in a biological sample, comprising: (a) a polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; and (b) a detection reagent.

In another embodiment, diagnostic kits are provided for detecting *Leishmania* infection in a biological sample, comprising: (a) a first amino acid sequence comprising an

epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; (b) a second amino acid sequence comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and (c) a detection reagent.

In still another embodiment, diagnostic kits are provided for identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis, comprising: (a) a polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and (b) a detection reagent.

In yet another related embodiment, the present invention provides diagnostic kits for identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis, comprising: (a) a first polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; (b) a second polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and (c) a detection reagent.

Within other aspects, this invention provides pharmaceutical compositions comprising a polypeptide containing an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications, and a physiologically acceptable carrier; and vaccines comprising a polypeptide as described above and an adjuvant.

In related aspects, DNA sequences encoding the above polypeptides, expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references

disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figures 1(a) and (b) show the sequence of a representative cDNA molecule encoding LcP0, along with the amino acid sequence encoded by the cDNA.

Figures 2(a), (b), and (c) depict the sequence of the full length K39 polypeptide.

Figures 3(a) and (b) present the DNA sequence of a representative cDNA encoding the full length K39 polypeptide.

Figure 4 illustrates the reactivity of LcP0. Figure 4(a) shows the reactivity with sera from normal individuals, Figure 4(b) shows the reactivity with asymptomatic patient sera and Figure 4(c) depicts the reactivity with sera from patients with acute visceral leishmaniasis.

Figure 5 illustrates the reactivity of a polypeptide containing the 17 C-terminal amino acids of LcP0 as compared to that of recombinant LcP0. Figure 5(a) shows the reactivity with sera from normal individuals, Figure 5(b) shows the reactivity with asymptomatic patient sera and Figure 5(c) depicts the reactivity with sera from patients with acute visceral leishmaniasis.

Figure 6 shows the reactivity of the 17 amino acid polypeptide evaluated in Figure 5. Figure 6(a) shows the reactivity with sera from normal individuals, Figure 6(b) shows the reactivity with sera from patients with acute visceral leishmaniasis and Figure 6(c) depicts the reactivity with asymptomatic patient sera.

Figure 7 illustrates the reactivity of the recombinant K39 polypeptide. Figure 7(a) shows the reactivity with sera from normal individuals, Figure 7(b) shows the reactivity with asymptomatic patient sera and Figure 7(c) depicts the reactivity with sera from patients with acute visceral leishmaniasis.

Figure 8 shows the reactivity of LcP0 together with K39, as compared to that of *Leishmania* lysate, with sera from normal, asymptomatic and visceral leishmaniasis patients.

Detailed Description of the Invention

As noted above, the present invention is generally directed to compounds and methods useful for detecting and protecting against *Leishmania* infection. The compounds of this invention generally comprise one or more antigenic epitopes of *Leishmania* proteins. In particular, polypeptides comprising an epitope of a *Leishmania chagasi* homolog of the eukaryotic acidic ribosomal P-protein family (referred to herein as LcP0) are disclosed. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including

full length proteins, wherein the amino acid residues are linked by covalent peptide bonds. The use of such LcP0 polypeptides for specifically detecting asymptomatic or subclinical leishmaniasis is also disclosed. In addition, the present invention discloses the use of epitopes from other *Leishmania* proteins, in combination with an epitope of LcP0, to diagnose *Leishmania* infection and to monitor the development of acute visceral leishmaniasis.

The compounds and methods of this invention also encompass variants of the recited polypeptides. As used herein, a "variant" is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that it retains the antigenic properties of the recited polypeptide. Such variants may generally be identified by modifying the polypeptide sequence as described below and evaluating the antigenic properties of the modified polypeptide using, for example, one or more of the assays described herein. A "conservative substitution" in the context of this invention is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. Preferred substitutions include changes between asp and glu, ala and glu, ala and ser, ala and thr, met and ser, and asn and ser. Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids, that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, the polypeptide may be conjugated to a linker or other sequence for ease of synthesis or to enhance binding of the polypeptide to a solid support.

In one aspect of the invention, polypeptides are provided comprising an epitope of the *Leishmania chagasi* acidic ribosomal antigen LcP0. A genomic DNA sequence encoding LcP0 is shown in Figures 1(a) and (b). A DNA molecule encoding LcP0 may be isolated by screening a *Leishmania chagasi* genomic expression library for clones that express antigens which react with pooled sera from *T. cruzi*-infected patients. This screen may be generally performed using methods known to those of ordinary skill in the art, such as methods described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y. 1989, which is incorporated herein by reference. Briefly, a bacteriophage expression library may be plated and transferred to filters. The filters may then be incubated with serum and a detection reagent. In the context of this invention, a "detection reagent" is any compound capable of binding to the antibody-antigen complex, which may then be detected by any of a variety of means known to those of ordinary skill in the art. Typical detection reagents contain a "binding agent," such as Protein A, Protein G, IgG or a lectin, coupled to a reporter group. Preferred reporter groups include,

but are not limited to, enzymes, substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. More preferably, the reporter group is horseradish peroxidase, which may be detected by incubation with a substrate such as tetramethylbenzidine or 2,2'-azino-di-3-ethylbenzthiazoline sulfonic acid. Plaques containing genomic DNA sequences that express a protein which binds to an antibody in the serum may be isolated and purified by techniques known to those of ordinary skill in the art. Appropriate methods may be found, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

Epitopes of LcP0 may generally be determined by generating polypeptides containing portions of the LcP0 sequence and evaluating the reactivity of the polypeptides with sera from *Leishmania*-infected individuals using, for example, an enzyme linked immunosorbent assay (ELISA). Suitable assays for evaluating reactivity of a polypeptide with *Leishmania*-infected sera are described in more detail below. Within such representative assays, portions of the LcP0 sequence that generate a signal that differentiates between positive and negative sera in a manner substantially similar to that of the full length LcP0 are considered to contain an epitope. In other words, a portion of LcP0 that contains an epitope will generate a signal indicating *Leishmania* infection in substantially all (*i.e.*, at least about 80%, and preferably at least about 90%) of the biological samples for which such infection would be indicated using the full length LcP0 and will generate a signal indicating the absence of *Leishmania* infection in substantially all of those samples that would be negative with the full length polypeptide. Portions of LcP0 containing at least the 17 C-terminal amino acids shown in Figures 1 (*i.e.*, residues 306-322) have generally been found to generate a signal in an ELISA that is substantially equivalent to that generated by the full length LcP0. Accordingly, polypeptides comprising at least the 17 C-terminal amino acids of LcP0 contain an epitope of LcP0, and such polypeptides (and variants thereof) are within the scope of this invention.

In a related aspect, combination polypeptides comprising epitopes of multiple *Leishmania* polypeptides are disclosed. A "combination polypeptide" is a polypeptide in which epitopes of different *Leishmania* peptides, or variants thereof, are joined though a peptide linkage into a single amino acid chain. The epitopes may be joined directly (*i.e.*, with no intervening amino acids) or may be joined by way of a linker sequence (*e.g.*, Gly-Cys-Gly) that does not significantly alter the antigenic properties of the epitopes.

In preferred embodiments, the combination polypeptide comprises an LcP0 epitope along with an epitope derived from the *Leishmania* K39 antigen (see Figures 2(a), (b) and (c) and U.S. Patent No. 5,411,865). More preferably, the K39 epitope is a K39 repeat unit antigen, having the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp

Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications.

The polypeptides of this invention may be generated using techniques well known to those of ordinary skill in the art. Polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, can be synthesized using, for example, the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied Biosystems, Inc., Foster City, CA. Thus, for example, the K39 repeat unit antigen, or portions thereof, may be synthesized by this method. Similarly, polypeptides comprising epitopes of LcP0, such as residues 306-322 of SEQ. ID NO:2, may be prepared using an automated synthesizer.

Alternatively, the polypeptides of this invention may be prepared by expression of recombinant DNA encoding the polypeptide in cultured host cells. Preferably, the host cells are *E. coli*, yeast, an insect cell line (such as *Spodoptera* or *Trichoplusia*) or a mammalian cell line, including (but not limited to) CHO, COS and NS-1. The DNA sequences expressed in this manner may encode naturally occurring proteins, such as LcP0 and K39, portions of naturally occurring proteins, or other variants of such proteins. Expressed polypeptides of this invention are generally isolated in substantially pure form. Preferably, the polypeptides are isolated to a purity of at least 80% by weight, more preferably, to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and affinity chromatography.

In another aspect of this invention, methods are disclosed for detecting and monitoring *Leishmania* infection, as well as for distinguishing among types of *Leishmania* infections, in individuals and blood supplies. In general, *Leishmania* infection may be detected in any biological sample that contains antibodies. Preferably, the sample is blood, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood or serum sample obtained from a patient or a blood supply. Briefly, *Leishmania* infection may be detected using one or more polypeptides containing one or more of the epitopes discussed above, or variants thereof. If multiple epitopes are employed, these epitopes may be present on one or more polypeptides. The polypeptide or polypeptides are then used to determine the presence or absence of antibodies to the polypeptide or polypeptides in the sample, relative to a predetermined cut-off value.

There are a variety of assay formats known to those of ordinary skill in the art for using a polypeptide to detect antibodies in a sample. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that binds to the antibody/polypeptide complex and contains a detectable reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized polypeptide after incubation of the polypeptide with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any material known to those of ordinary skill in the art to which the polypeptide may be attached. For example, the support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptide may be bound to the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 μ g, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen. Nitrocellulose will bind approximately 100 μ g of protein per cm^2 .

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to a support having an appropriate polymer coating

using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (see, e.g., Pierce Immunotechnology Catalog and Handbook (1991) at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

Once the polypeptide is immobilized on the support, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20TM (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody (if present in the sample) is allowed to bind to the antigen. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to permit detect the presence of antibody within a *Leishmania*-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20TM. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many sources (*e.g.*, Zymed Laboratories, San Francisco, CA and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-*Leishmania* antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is preferably the average mean signal obtained when the immobilized polypeptide is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive (*i.e.*, reactive with the polypeptide). In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, p. 106-7 (Little Brown and Co., 1985). Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the antigen is immobilized on a membrane such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (*e.g.*, protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the

membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of *Leishmania* antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (e.g., one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only.

In one aspect of the invention, the assays discussed above may be used to specifically detect asymptomatic or sub-clinical leishmaniasis. In this aspect, antibodies in the sample may be detected using a polypeptide comprising LcP0 or an epitope thereof. Preferably, the polypeptide comprises amino acids 306-322 of SEQ ID NO:2. It has been found in the present invention that polypeptides comprising at least this C-terminal portion of LcP0 generate a positive result with sera from less than 35% of patients with acute visceral leishmaniasis, but generate a positive result with sera from more than 95% patients having asymptomatic or sub-clinical leishmaniasis. Accordingly, LcP0, and antigenic portions thereof, may be used to specifically identify patients with asymptomatic or sub-clinical leishmaniasis.

In another aspect, both asymptomatic/sub-clinical and acute visceral leishmaniasis may be detected. In this aspect, an LcP0 epitope is combined with a second *Leishmania* epitope that detects the presence of acute visceral leishmaniasis. Preferably, the second epitope comprises at least one repeat unit of the K39 antigen, the sequence of which is provided in Figures 2(a), (b) and (c) and SEQ ID NO:3. In one such embodiment, the K39 antigen comprises the repeat unit antigen having the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala. In another such preferred embodiment, the K39 epitope is present within a recombinant K39 polypeptide, which comprises amino acids 1-955 of SEQ ID NO:3. The use of variants of the

above sequences, that differ only in conservative substitutions and/or modifications, is also preferred.

Preferably, the LcP0 and K39 antigens are immobilized by adsorption to a solid support such as a well of a microtiter plate or a membrane, as described above, in roughly similar amounts such that the total amount of polypeptide in contact with the support ranges from about 10 ng to about 100 µg. The remainder of the steps in the assay may generally be performed as described above. It will be readily apparent to those of ordinary skill in the art that, by combining LcP0 and K39 polypeptides with other polypeptides that can detect cutaneous and mucosal leishmaniasis, the polypeptides disclosed herein may be used in methods that detect all types of leishmaniasis.

In another aspect of the invention, patients with asymptomatic or subclinical VL whose disease is likely to progress to acute visceral leishmaniasis may be distinguished from infected patients whose disease is not likely to progress. Such progression may occur within a year (and typically within 5-12 months) for subclinical disease, or within many years in the case of asymptomatic patients. This determination may be made using any of several approaches. In one embodiment, the assay is performed using a polypeptide that comprises at least one repeat unit of the K39 antigen, without the use of an LcP0 epitope. Preferably, the polypeptide comprises the K39 repeat unit antigen described above. While the K39 repeat unit antigen generates a positive result (relative to the predetermined cut-off value) when reacted with sera from more than 97% of patients with acute visceral leishmaniasis, only a relatively small percentage (around one third) of patients with asymptomatic leishmaniasis react with this antigen. Those sera that do react are likely to indicate infections that are in the process of progression, or are likely to progress, to acute visceral leishmaniasis (or infections that are in remission or responding to treatment, which may be distinguished based on patient history).

In another embodiment, the assay is separately performed with LcP0, or an epitope thereof, and with a polypeptide that comprises at least one repeat unit of the K39 antigen. In this embodiment, the optical density (OD) obtained in the assay using the LcP0 epitope is compared to the value obtained using the K39 polypeptide. A relatively high OD in the assay using the LcP0 epitope, along with a relatively low OD in the assay using the K39 polypeptide indicates an asymptomatic or subclinical infection that is not likely to progress to acute visceral leishmaniasis. On the other hand, a relatively high OD in the assay using the K39 polypeptide, along with a relatively low OD in the assay using the LcP0 epitope indicates an asymptomatic or subclinical infection that is likely to progress to acute visceral leishmaniasis (or in remission or responding to treatment). Those asymptomatic or subclinical patients for whom both values are relatively high are likely to be in the process of developing acute visceral leishmaniasis (or in the process of recovering from infection). In each case, the

direction of the disease (*i.e.*, progression or remission) may be determined using the patient's history.

In another embodiment, asymptomatic or subclinical patients that are likely to develop acute visceral leishmaniasis may be identified using separate LcP0 and K39 assays (as described above) that are performed over a period of time. For example, the assays may be performed every 1-6 months for a period of months or years. Asymptomatic or subclinical patients that are likely to remain asymptomatic or subclinical will generally have sera that shows a high reactivity with LcP0 and a low reactivity with the K39 polypeptide, as discussed above, at each time point. However, patients that are progressing toward acute visceral leishmaniasis will show an increase in the reactivity with the K39 polypeptide and a decrease in the reactivity with LcP0 over the time period of the assays. By monitoring an individual patient in this manner, the development of acute visceral leishmaniasis may be identified before other symptoms become apparent. This early identification allows selective treatment of only those asymptomatic patients that are predisposed to develop a more serious form of the disease.

In another aspect of this invention, immobilized LcP0 polypeptides may be used to purify antibodies that bind to LcP0. Such antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Land, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising a LcP0 polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). In this step, the polypeptide may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed.

For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In this process, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. One or more LcP0 polypeptides may be used in the purification process in, for example, an affinity chromatography step.

Monospecific antibodies that bind to an LcP0 polypeptide may be used, for example, to detect *Leishmania* infection in a biological sample using one of a variety of immunoassays, which may be direct or competitive. Briefly, in one direct assay format, a monospecific antibody may be immobilized on a solid support (as described above) and contacted with the sample to be tested. After removal of the unbound sample, a second monospecific antibody, which has been labeled with a reporter group, may be added and used to detect bound antigen. In an exemplary competitive assay, the sample may be combined with the monoclonal or polyclonal antibody, which has been labeled with a suitable reporter group. The mixture of sample and antibody may then be combined with polypeptide antigen immobilized on a suitable solid support. Antibody that has not bound to an antigen in the sample is allowed to bind to the immobilized antigen, and the remainder of the sample and antibody is removed. The level of antibody bound to the solid support is inversely related to the level of antigen in the sample. Thus, a lower level of antibody bound to the solid support indicates the presence of *Leishmania* in the sample. Other formats for using monospecific antibodies to detect *Leishmania* in a sample will be apparent to those of ordinary skill in the art, and the above formats are provided solely for exemplary purposes.

In another aspect of this invention, vaccines and pharmaceutical compositions are provided for the prevention of *Leishmania* infection, and complications thereof, in a mammal, preferably a human or dog. Pharmaceutical compositions generally comprise one or more polypeptides, containing one or more epitopes of *Leishmania* proteins, and a physiologically acceptable carrier. The vaccines comprise one or more of the above polypeptides and an adjuvant, for enhancement of the immune response.

Routes and frequency of administration and polypeptide doses will vary from individual to individual and may parallel those currently being used in immunization against other protozoan infections. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 4 doses may be administered for a 2-6 week period. Preferably, two doses are administered, with the second dose 2-4 weeks later than the first. A suitable dose is an amount of polypeptide that is effective to raise antibodies in a treated mammal that are sufficient to protect the mammal from *Leishmania* infection for a period of time. In general, the amount of polypeptide present in a dose ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the animal, but will typically range from about 0.01 mL to about 5 mL for 10-60 kg animal.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention.

Any of a variety of adjuvants may be employed in the vaccines of this invention to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune response, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis*. Such adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ).

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1
Preparation of LcP0

This Example illustrates the isolation of a genomic DNA sequence encoding LcP0 and the preparation of LcP0.

A genomic expression library was constructed with sheared DNA of *L. chagasi* (MHOM/BR/82/BA-2,C1) in bacteriophage λ ZAPII (Stratagene, La Jolla, California). The library was screened and pBSK(-) phagemid sequences were excised according to the manufacturer's protocols. For this screen, serum samples from 5 *T. cruzi*-infected individuals were pooled and anti-*E. coli* reactivity was removed by adsorption. One clone, containing an approximately 3 kb insert, was isolated (pLcP0). Expression of pLcP0 produced a recombinant fusion protein of approximately 42 kD, of which about 4 kD represented a plasmid fusion sequence.

The DNA sequence (shown in Figures 1(a) and (b)) contained a single open reading frame (nucleotides 1-966) encoding 322 amino acids with a predicted molecular weight of 34,600. Rabbit anti-serum against purified recombinant LcP0 was used to probe immunoblots of *L. chagasi* promastigote lysate. The anti-serum reacted specifically to a 37 kD antigen present in the promastigotes. These results suggest that the recombinant DNA sequence contained the entire coding region of LcP0.

To further verify that the LcP0 genomic clone contained the full-length protein sequence, a cDNA library was screened with the LcP0 clone. Briefly, poly(A)⁺ RNA was purified from total *L. chagasi* (MHOM/BR/84/Jonas) promastigote RNA, using standard protocols. A cDNA expression library was constructed with the poly(A)⁺ RNA, using the ZAP-cDNA unidirectional cloning kit (Stratagene, La Jolla, California). This library was screened as described above for the genomic DNA library, and a 1.2 kb cDNA clone was isolated. Partial sequence analysis of the 5' and 3' portions revealed that it encoded a full-length LcP0 insert. The sequence contained the last 8 nucleotides of the *trans*-spliced leader sequence found on the 5' end of all trypanosome nuclearly-encoded transcripts, followed by a short (29 nucleotide) 5' untranslated leader sequence. Partial sequencing of the 3' portion of the cDNA revealed an open reading frame and a stop codon (TAA) followed by a 203 nucleotide 3'-untranslated portion terminating in a stretch of poly(A) residues. The 5' and 3' ends of the cDNA were present in the genomic clone. Accordingly, the genomic LcP0 clone encodes the complete LcP0 protein.

Full-length LcP0 was produced and purified from *E. coli* transformed with an expression vector containing the genomic clone pLcP0. Purification to homogeneity was

accomplished by preparative SDS-PAGE, followed by excision and electroelution of the recombinant antigen. The SDS-PAGE was performed by loading expressed protein onto a 12% polyacrylamide gel in sample buffer (50 mM Tris-HCl, 1% SDS, 10% glycerol, 2% 2-mercaptoethanol, 0.01% bromphenol blue) and running according to standard procedure. A section of the gel was transferred to nitrocellulose for immunoblotting with patient serum for band identification. Bands of interest were excised and gel slices were diced into 2-3 mm cubes and soaked overnight at 4°C in 2% SDS, 0.4 M NH₄HCO₃ and 0.1% dithiothreitol. The gel pieces and soaking buffer were then placed into an electro-eluter (Bio-Rad Laboratories, Richmond, CA). Elution occurred for 6-7 hours at 10 mA per tube in 0.5 M NH₄HCO₃, 0.1% SDS. The eluted fractions were dialyzed against 0.01 M NH₄HCO₃, 0.02% SDS for 24 hours, followed by dialysis against a minimum of 100 volumes of PBS, pH 7.4 for 3-5 days with two buffer changes per 24 hours. All dialysis was done at 4 °C. Eluted samples were assayed for protein content using the Pierce assay (Pierce Chemical Co., Rockford, IL) and checked for purity on SDS-PAGE minigels with silver staining (Bio-Rad Laboratories, Richmond, CA).

Example 2

Detection of Asymptomatic *Leishmania* using LcP0

This Example illustrates the detection of *Leishmania* infection using LcP0, prepared as described in Example 1, in an ELISA format.

The ELISA assays were performed as follows. Plastic 96-well plates (Probind, Falcon Plastics, Cockeysville, MD) were coated with 250 ng of LcP0, diluted to 50 µl with 0.05 M carbonate buffer (pH 9.6), and incubated overnight. Sensitized wells were washed with 0.01 M phosphate buffered saline (pH 7.2) containing 0.3% Tween 20™ (PBS/T). Positive control, negative control, and unknown serum samples were diluted 1:50 in PBS/T, and 50 µl was added to each well. After 30 minutes of incubation at room temperature, wells were washed six times with PBS/T. Fifty µl of protein-A peroxidase (Zymed Laboratories, San Francisco, CA), diluted in PBS/T was added and the plates were incubated as described above. Wells were washed eight times with PBS/T and 100 µl of 2,2'-azino-di-3-ethylbenzethiazoline sulfonic acid (ABTS) substrate solution (50 µl of 50 X ABTS, 50 µl of 1.5% H₂O₂, 2.5 ml of 0.1 M citrate buffer (pH 4.1), Zymed Laboratories, San Francisco, CA) was added. After 15 minutes at room temperature, the enzymatic reaction was stopped by adding 100 µl of 10% sodium dodecylsulfate. A₄₀₅ values were determined with an ELISA reader (Titertek Multiskan, Flow Laboratories, McLean, VA). The cut-off value was determined for each test by calculating the mean of negative sera plus three standard deviations.

Individuals in Brazil with asymptomatic leishmaniasis (AL) or acute visceral leishmaniasis (AVL) were identified based on serology (e.g., IFAT or IHA immunofluorescence or hemagglutination), clinical symptoms (e.g., malaise, diarrhea, splenomegaly and hepatomegaly) and whole lysate ELISA. Of 21 serum samples from patients with AL, all (i.e., 100%) tested positive using the above assay. However, of 31 serum samples from patients with AVL, only 9 (i.e., 29%) were positive. In addition, 44 normal serum samples (from unexposed individuals in Seattle, WA) were assayed. All 44 (i.e., 100%) of the normal samples were negative. When the assay was performed using crude lysate, all 44 normal samples were negative, all 21 AL samples were positive, and 28 out of 31 AVL samples were positive.

These results are depicted in Figure 4. Figure 4(a) shows the distribution of absorbance values at 405 nm for the normal sera assayed with LcP0. In Figure 4(b), the distribution for sera from individuals with AL is presented and, in Figure 4(c), the distribution for sera from individuals with AVL is shown.

These results demonstrate that LcP0 may be used to detect asymptomatic leishmaniasis, with a very low incidence of false positive results in normal individuals.

Example 3

Detection of Asymptomatic *Leishmania* using the C-terminal Epitope of LcP0

This Example illustrates the detection of *Leishmania* infection using a polypeptide containing the 17 C-terminal amino acids of LcP0 (i.e., residues 306-322) in an ELISA format.

The polypeptide was synthesized on an ABI 430A peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. Cleavage of the polypeptide from the solid support was carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the polypeptide was precipitated in cold methyl-t-butyl-ether. The pellet was then dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) was used to elute the peptides. Following lyophilization of the pure fractions, the polypeptide was characterized using electrospray mass spectrometry and by amino acid analysis. The synthesized C-terminal polypeptide had the sequence Glu-Glu-Pro-Glu-Glu-Ser-Asp-Glu-Asp-Asp-Phe-Gly-Met-Gly-Gly-Leu-Phe (i.e., residues 306-322 of the amino acid sequence of Figures 2(a), (b) and (c) and SEQ ID NO:3).

The ELISA assays were performed as described in Example 2, using either 250 ng of LcP0 or 1 µg of the above C-terminal polypeptide. AL, AVL and normal serum

samples were assayed. Of 20 serum samples from patients with AL, all tested positive in the assay using full length LcP0, and 19 (*i.e.*, 95%) tested positive with the C-terminal polypeptide. Of 28 serum samples from patients with AVL, 20 (*i.e.*, 28%) were positive. In addition, all 45 (*i.e.*, 100%) of the normal samples were negative.

These results are depicted in Figure 5. Figure 5(a) shows the distribution of absorbance values at 405 nm for the normal sera assayed with LcP0 and the C-terminal polypeptide. In Figure 5(b), the distribution for sera from individuals with AL is presented and, in Figure 5(c), the distribution for sera from individuals with AVL is shown.

These results demonstrate that a polypeptide containing the 17 C-terminal amino acids of LcP0 contains a major epitope and may be used to detect asymptomatic leishmaniasis, with a very low incidence of false positive results in normal individuals.

Example 4

Detection of *Leishmania* Infection in Serum with K39

This Example demonstrates the detection of *Leishmania* infection using a recombinant K39 polypeptide.

In one experiment, the assays were performed in an ELISA format, as described in Example 2, except that the wells were coated with 100 ng of recombinant K39 polypeptide having the amino acid sequence shown in Figures 2(a), (b) and (c).

In this experiment, the AL, AVL and normal serum samples described in Example 2 were assayed. Of 21 serum samples from patients with AL, 17 (*i.e.*, 33%) tested positive in the assay. However, of 31 serum samples from patients with AVL, 30 (*i.e.*, 97%) were positive. In addition, all 44 (*i.e.*, 100%) of the normal samples were negative.

These results are depicted in Figure 6. Figure 6(a) shows the distribution of absorbance values at 405 nm for the normal sera assayed with K39. In Figure 6(b), the distribution for sera from individuals with AVL is presented and, in Figure 6(c), the distribution for sera from individuals with AL is shown.

These results demonstrate that K39 detects acute visceral leishmaniasis, with a very low incidence of false positive results in normal individuals, but that K39 is not sensitive for detecting asymptomatic leishmaniasis. In addition, Figures 4-6, and the results described in Example 2 and this Example, indicate that nearly all patients with AVL or AL (*i.e.*, 60 out of 61) tested positive with at least one of LcP0 and K39. This indicates that the two antigens, used in combination, would detect both types of leishmaniasis.

To further illustrate this point, Table 1, below, shows the absorbance values for the 21 AL samples and the 31 AVL samples tested with LcP0 and K39.

Table 1
Reactivity of Serum Samples with LcPO and K39 Antigens

Serum	Absorbance (405 nm)	
	K39	LcPO
AL		
01SP	0.07	1.47
02SP	0.04	0.64
04SP	0.1	>3.0
05SP	0.08	2.07
06SP	0.06	1.95
07SP	0.33	2.18
08SP	0.08	2.02
09SP	0.10	>3.0
10SP	0.62	2.49
11SP	0.08	>3.0
12SP	0.88	1.77
13OL	0.10	2.07
14OL	0.07	2.65
15OL	0.03	2.19
16OL	0.12	1.35
17OL	>3.0	0.78
19OL	0.11	1.41
21OL	0.29	1.84
22OL	2.42	>3.0
25aPK	0.11	0.35
27PK	0.92	0.98
AVL		
K8	2.31	0.12
K22	1.47	0.08
K36	2.52	0.16
K39	2.60	0.39
K49	2.50	1.94
K52	>3.0	0.25
K2	>3.0	2.48
K7	>3.0	0.17

K11	1.58	0.07
K18	>3.0	0.28
K24	>3.0	0.17
K26	2.60	0.08
K33	2.22	0.11
K41	>3.0	0.24
K46	>3.0	0.23
K50	2.31	0.01
K54	2.70	0.22
K61	>3.0	1.14
K68	>3.0	0.12
K70	>3.0	0.08
K71	>3.0	0.30
K74	>3.0	0.17
K2	>3.0	0.61
K50	1.27	0.02
K68	>3.0	0.60
139177	0.09	0.13
139178	0.38	0.13
139180	0.28	0.31
139181	>3.0	0.93
139182	0.66	0.62
139183	1.97	1.44

As shown in the above table, most samples with very low absorbance values when assayed with one antigen had high absorbances when assayed with the other antigen. Accordingly, LcP0 and K39 are complementary and, together, form a sensitive assay for monitoring disease progression from asymptomatic/subclinical disease to acute visceral disease, or vice versa.

Example 5

Detection of *Leishmania* Infection in Serum with LcP0 in Combination with K39

This Example demonstrates the detection of *Leishmania* infection using LcP0 in combination with a recombinant K39 polypeptide.

Assays were performed as described in Example 2 except that, in addition to the LcP0, wells were coated with 100 ng of recombinant K39. In a parallel experiment, the assays were performed using *Leishmania* lysate as the antigen. The same AL, AVL and

normal serum samples described in Example 2 were assayed. Of the 21 serum samples from patients with AL, 21 (*i.e.*, 100%) tested positive in the assay. Of 31 samples from patients with AVL, 30 (*i.e.*, 97%) were positive. All (*i.e.*, 100%) of the normal samples were negative.

The absorbance values for representative assays using LcPO/K39 or *Leishmania* lysate are shown in Table 2 below.

Table 2
Reactivity of Serum Samples With *Leishmania* Lysate and
K39/LcPO Antigens

SAMPLE ID	Lc LYSATE	K39+LcPO	STATUS
01-SP	1.545	1.148	ASYMPTOMATIC
02-SP	1.174	0.34	ASYMPTOMATIC
03-SP	1.263	0.168	ASYMPTOMATIC
04-SP	2.533	2.521	ASYMPTOMATIC
06-SP	1.478	1.556	ASYMPTOMATIC
07-SP	1.365	1.701	ASYMPTOMATIC
08-SP	1.192	1.369	ASYMPTOMATIC
220L	1.669	3	ASYMPTOMATIC
10SP	1.651	1.951	ASYMPTOMATIC
140L	1.192	2.061	ASYMPTOMATIC
150L	0.863	1.461	ASYMPTOMATIC
160L	2.104	1.067	ASYMPTOMATIC
170L	3	3	ASYMPTOMATIC
180L	2.032	1.264	ASYMPTOMATIC
190L	1.586	0.908	ASYMPTOMATIC
210L	1.52	1.572	ASYMPTOMATIC
139189	0.348	0.431	AVL
139197	0.481	2.455	AVL
139199	1.235	2.601	AVL
139200	2.022	0.792	AVL
139202	1.182	0.263	AVL
139204	1.375	0.964	AVL
139205	3	3	AVL

139206	2.209	3	AVL
K18	0.95	3	AVL
K26	1.298	3	AVL
K41	3	3	AVL
K52	3	3	AVL
K54	3	3	AVL
K74	2.509	3	AVL
K71	3	3	AVL
K75	2.118	3	AVL
4/15/93-2	0.003	0.004	NORMAL
4/15/93-3	0.001	0.007	NORMAL
4/15/93-4	0.001	0.004	NORMAL
6/15/93-3	0	0.002	NORMAL
6/15/93-2	0.001	0.004	NORMAL
2/9/94-4	0.003	0.003	NORMAL
2/9/94-3	0.003	0.001	NORMAL
2/9/94-2	0.005	0.006	NORMAL
9/9/93-2	0.17	0.046	NORMAL
7/22/94-4	0.034	0.049	NORMAL
7/22/94-3	0.055	0.055	NORMAL
7/22/94-2	0.034	0.066	NORMAL
7/22/94-1	0.029	0.051	NORMAL
3/25/94-4	0.035	0.023	NORMAL
3/25/94-3	0.02	0.021	NORMAL

The absorbance values provided above are depicted in Figure 7, which shows the distribution of absorbance values at 405 nm for assays performed using representative sera from normal AL and AVL patients. The results of this experiment demonstrate that LcP0, in combination with K39, detects asymptomatic and acute visceral leishmaniasis, with a very low incidence of false positive results in normal individuals.

Example 6

Non-reactivity of LcP0 and K39 with Sera from Patients with Mucosal or Cutaneous Leishmaniasis

This Example shows that LcP0 and K39 are specific for asymptomatic/subclinical and visceral leishmaniasis, respectively. Assays were generally performed as described in Examples 4 and 5, except that the sera assayed was from patients with mucosal or cutaneous leishmaniasis, as diagnosed by clinical symptoms. Fourteen samples from patients with each form of the disease were assayed with LcP0, K39 and a combination of LcP0 and K39, as well as with *Leishmania* lysate. With each set of 14 samples, a positive control (*i.e.*, a sample obtained from a patient infected with *Leishmania*) and a blank with no antibodies were assayed, in addition to eight samples from normal individuals.

The absorbance values are shown below in Table 3.

Table 3
Non-reactivity of LcP0 and K39 With Sera From
Patients With Mucosal and Cutaneous Leishmaniasis

Serum	Lc lysate	K39+LcP0	K39	LcP0
MUCOSAL				
M1	0.312	0.033	0.03	0.011
M2	0.11	0.036	0.015	0.029
M3	0.37	0.054	0.003	0.055
M4	0.109	0.039	0.036	0.007
M5	0.586	0.02	0.009	0.016
M6	0.116	0.038	0.025	0.017
M7	0.245	0.156	0.099	0.067
M8	0.09	0.078	0.05	0.047
M9	0.046	0.026	0.026	0.003
M10	0.343	0.042	0.013	0.033
M11	0.327	0.34	0.009	0.346
M12	0.035	0.014	0.003	0.008
M13	0.131	0.031	0.026	0.007
M14	0.282	0.014	0.006	0.006
positive control	0.444	0.543	0.545	0.114
No Ab	0.001	0.002	-0.002	-0.001
CUTANEOUS				

C1	0.06225	0.01255	0.00725	0.0033
C2	0.1602	0.01225	0.00325	0.0083
C3	0.1402	0.03925	0.02625	0.0143
C4	0.1932	0.01725	0.01325	0.0053
C5	0.1672	0.04825	0.01725	0.0283
C6	0.1672	0.02825	0.01925	0.0213
C7	0.09025	0.07425	0.07225	0.0103
C8	0.05425	0.01725	0.00725	0.0103
C9	0.0803	0.05525	0.05425	0.007
C10	0.2702	0.04825	0.03225	0.019
C11	0.4882	0.3782	0.1992	0.297
C12	0.5632	0.5352	0.5312	-0.002
C13	0.0263	0.01425	0.00425	0.005
C14	0.0293	0.01525	0.01525	0.003
positive control	0.4132	0.5732	0.5362	0.096
No Ab	0.0043	0.00025	-0.00275	-0.002
<hr/>				
NORMAL				
6/8/93-1	0.018	0.01	0.005	0.005
6/8/93-2	0.03	0.01	0.005	0.006
6/8/93-3	0.016	0.02	0.012	0.003
8/31/93-3	0.018	0.014	0.002	0.01
8/31/93-4	0.003	0.005	-4E-09	0.005
9/10/93-2	0.006	0.004	-4E-09	0.002
9/10/93-3	0.005	-3.7E-09	-0.001	-4E-09
4/15/93-2	0.014	0.043	0.037	0.009
1/12/93	0.0023	0.00725	0.0013	0.0003
3/2/94-4	0.0013	0.00025	-0.0028	0.0003
3/2/94-3	-0.002	-0.00275	-0.0048	-0.006
3/2/94-2	0.0003	0.00025	-0.0008	-0.008
3/2/94-1	0.0043	0.00225	-0.0048	0.0033
4/28/94-3	-0.002	-0.00375	-0.0058	-0.012
4/28/94-2	0.0083	0.00225	-0.0028	0.0003
4/15/93-2	0.0123	0.04325	0.0333	0.0023

Of the normal serum samples assayed, no positive results were obtained using either LcP0 or K39, or a combination of the two. Of the 14 mucosal samples, one tested positive with LcP0 and LcP0/K39, and none were positive with K39 alone. When the 14 cutaneous samples were assayed, one tested positive with each of LcP0 and K39, and two were positive with the LcP0/K39 combination. These results demonstrate that assays performed using LcP0 and/or K39 detect only a small percentage of mucosal and cutaneous manifestations of leishmaniasis.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT: Reed, Steven G.

(ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR
DIAGNOSIS OF LEISHMANIASIS

10

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:

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- (E) COUNTRY: USA
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20

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

30

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US
- (B) FILING DATE: 21-DEC-1995
- (C) CLASSIFICATION:

35

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Kadlecak, Ann T.
- (B) REGISTRATION NUMBER: P-39,244
- (C) REFERENCE/DOCKET NUMBER: 210121.407

40

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- (C) TELEX: 3723836 SEEDANDBERRY

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- 5
(A) LENGTH: 1202 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 30..998

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATAGCCAAGG CTATTGCAAG TCTCACAAAG ATG CCG TCT ATC ACC ACT
GCC AAG

20

Met Pro Ser Ile Thr Thr

Ala Lys

1

5

25 CGC GAG TAC GAG GAG CGC CTC GTC GAC TGC CTG ACC AAG TAC
AGC TGC

101

Arg Glu Tyr Glu Glu Arg Leu Val Asp Cys Leu Thr Lys Tyr
Ser Cys

10

15

20

30 GTG CTG TTC GTG GGC ATG GAC AAC GTC CGC TCG CAG CAG GTG
CAC GAT

149

Val Leu Phe Val Gly Met Asp Asn Val Arg Ser Gln Gln Val
His Asp

25

30

35

35 40

GTG CGC CGT GGC TGT CGC GGC AAG GCC GAG TTC ATT ATG GGC
AAG AAG

197

40 Val Arg Arg Gly Cys Arg Gly Lys Ala Glu Phe Ile Met Gly
Lys Lys

	45		50
	55		
5	ACG CTG CAG GCG AAG ATC GTG GAG AAG CGC GCG CAA GCC AAG GAC GCG		
	Thr Leu Gln Ala Lys Ile Val Glu Lys Arg Ala Gln Ala Lys		
	Asp Ala		
	60	65	70
10	AGC CCC GAG GCG AAG CCT TTC AAC GAT CAG TGT GAG GAG TAC AAC CTG		
	Ser Pro Glu Ala Lys Pro Phe Asn Asp Gln Cys Glu Glu Tyr		
	Asn Leu		
	75	80	85
15	CTG AGC GGC AAC ACC GGC CTC ATC TTC ACT AAC AAC GCT GTC CAG GAG		
	Leu Ser Gly Asn Thr Gly Leu Ile Phe Thr Asn Asn Ala Val		
	Gln Glu		
20	90	95	100
	ATC ACC TCT GTG CTT GAC GGC CAC CGC GTG AAG GCC CCG GCG CGT GTC		
	Ile Thr Ser Val Leu Asp Gly His Arg Val Lys Ala Pro Ala		
25	Arg Val		
	105	110	115
	120		
30	GGA GCG ATT CCG TGC GAC GTG GTT GTG CCT GCT GGC AGC ACC GGC ATG		
	437		
	Gly Ala Ile Pro Cys Asp Val Val Val Pro Ala Gly Ser Thr		
	Gly Met		
	125		130
	135		
35	GAG CCG ACC CAG ACG TCC TTC CAG GCG CTG AAC ATT GCG ACG AAG		
	485		
	Glu Pro Thr Gln Thr Ser Phe Phe Gln Ala Leu Asn Ile Ala		
	Thr Lys		
40	140	145	150

ATT GCC AAG GGT ATG GTG GAG ATC GTG ACG GAG AAG AAG GTG
 CTG AGC 533

Ile Ala Lys Gly Met Val Glu Ile Val Thr Glu Lys Lys Val
 5 Leu Ser

155	160	165
-----	-----	-----

GTC GGC GAC AAG GTG GAC AAC TCG ACG GCG ACG CTG CTG CAA
 AAG CTG 581

10 Val Gly Asp Lys Val Asp Asn Ser Thr Ala Thr Leu Leu Gln
 Lys Leu

170	175	180
-----	-----	-----

AAC ATC AGC CCG TTC TAC TAC CAG GTG AAT GTG CTG TCC GTG
 15 TGG GAC 629

Asn Ile Ser Pro Phe Tyr Tyr Gln Val Asn Val Leu Ser Val
 Trp Asp

185	190	195
-----	-----	-----

200

CGC GGT GTG CTG TTC ACC CGC GAG GAC CTC ATG ATG ACG GAG
 GAC ATG 677

Arg Gly Val Leu Phe Thr Arg Glu Asp Leu Met Met Thr Glu
 Asp Met

205	210	
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215

GTG GAG AAG ATG CTG ATG GAA GGC CTG AGC AAC GTT GCG GCG
 ATG GCG 725

30 Val Glu Lys Met Leu Met Glu Gly Leu Ser Asn Val Ala Ala
 Met Ala

220	225	230
-----	-----	-----

CTG GGT GCT GGC ATC CCG ACG TCT TCG ACG ATT GGC CCG ATG
 35 CTG GTG 773

Leu Gly Ala Gly Ile Pro Thr Ser Ser Thr Ile Gly Pro Met
 Leu Val

235	240	245
-----	-----	-----

40 GAC GCC TTC AAG AAC CTG CTG GCT GTC TCC GTG GCG ACC TCG
 TAC GAG 821

	Asp Ala Phe Lys Asn Leu Leu Ala Val Ser Val Ala Thr Ser		
	Tyr Glu		
250	255	260	
5	TTC GAG GAG CAC AAC GGC AAG GAG CTG CGC GAG GCC GCG ATC AAC GGC	869	
	Phe Glu Glu His Asn Gly Lys Glu Leu Arg Glu Ala Ala Ile		
	Asn Gly		
265	270	275	
10	280		
	CTG CTG GCC GGC TCT GGC TCG GCT GCT GCG GAG CCC GCC GCT GCC GCG	917	
	Leu Leu Ala Gly Ser Gly Ser Ala Ala Ala Glu Pro Ala Ala		
15	Ala Ala		
	285	290	
	295		
20	CCG GCC GCC CCT AGC GCT GCT GCC AAG GAG GAG CCG GAG GAG AGC GAC	965	
	Pro Ala Ala Pro Ser Ala Ala Ala Lys Glu Glu Pro Glu Glu		
	Ser Asp		
	300	305	310
25	GAG GAC GAC TTC GGC ATG GGC GGT CTC TTC TAA GCGACTCGCT ATCCGCCACC	1018	
	Glu Asp Asp Phe Gly Met Gly Gly Leu Phe *		
	315	320	
30	CAGCACCGTC GAGTGTTCGT GCGTTCGCAT GGTGGACAGT GGCGAGCGTG TGATGCCCTT	1078	
	GGATCATCAG GAAGCAAATC TCTCCCTTTC TCTGGGTGTT CTTCGTTCT TCTTCATTT	1138	
35	GTTTTGATC GCCGTGGCGC TGCGGCGATC GCTCAGTTCT TATTTTCGAT CAACCAACAA	1198	
	CGAA	1202	

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 322 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Ser Ile Thr Thr Ala Lys Arg Glu Tyr Glu Glu Arg
Leu Val

15 1 5 10
15 15

Asp Cys Leu Thr Lys Tyr Ser Cys Val Leu Phe Val Gly Met
Asp Asn

20 20 25 30
20

Val Arg Ser Gln Gln Val His Asp Val Arg Arg Gly Cys Arg
Gly Lys

35 35 40 45

25 Ala Glu Phe Ile Met Gly Lys Lys Thr Leu Gln Ala Lys Ile
Val Glu

50 55 60

30 Lys Arg Ala Gln Ala Lys Asp Ala Ser Pro Glu Ala Lys Pro
Phe Asn

65 65 70 75
80

35 Asp Gln Cys Glu Glu Tyr Asn Leu Leu Ser Gly Asn Thr Gly
Leu Ile

85 85 90
95

Phe Thr Asn Asn Ala Val Gln Glu Ile Thr Ser Val Leu Asp
Gly His
100 105 110

5 Arg Val Lys Ala Pro Ala Arg Val Gly Ala Ile Pro Cys Asp
Val Val
115 120 125

10 Val Pro Ala Gly Ser Thr Gly Met Glu Pro Thr Gln Thr Ser
Phe Phe
130 135 140

15 Gln Ala Leu Asn Ile Ala Thr Lys Ile Ala Lys Gly Met Val
Glu Ile
145 150 155

20 160

Val Thr Glu Lys Lys Val Leu Ser Val Gly Asp Lys Val Asp
Asn Ser
165 170

25 175

Thr Ala Thr Leu Leu Gln Lys Leu Asn Ile Ser Pro Phe Tyr
Tyr Gln
180 185 190

30 Val Asn Val Leu Ser Val Trp Asp Arg Gly Val Leu Phe Thr
Arg Glu
195 200 205

Asp Leu Met Met Thr Glu Asp Met Val Glu Lys Met Leu Met
Glu Gly
210 215 220

35 Leu Ser Asn Val Ala Ala Met Ala Leu Gly Ala Gly Ile Pro
Thr Ser

225	230	235
240		
Ser Thr Ile Gly Pro Met Leu Val Asp Ala Phe Lys Asn Leu		
5	Leu Ala	
	245	250
255		
Val Ser Val Ala Thr Ser Tyr Glu Phe Glu Glu His Asn Gly		
10	Lys Glu	
	260	265
		270
Leu Arg Glu Ala Ala Ile Asn Gly Leu Leu Ala Gly Ser Gly		
	Ser Ala	
15	275	280
		285
Ala Ala Glu Pro Ala Ala Ala Pro Ala Ala Pro Ser Ala		
	Ala Ala	
	290	295
20		300
Lys Glu Glu Pro Glu Glu Ser Asp Glu Asp Asp Phe Gly Met		
	Gly Gly	
305	310	315
320		
25		
	Leu Phe	

(2) INFORMATION FOR SEQ ID NO:3:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 955 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- 35 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

5 Met His Pro Ser Thr Val Arg Arg Glu Ala Glu Arg Val
 Lys Val Ser
 1 5 10
 15

10 Val Arg Val Arg Pro Leu Asn Glu Arg Glu Asn Asn Ala
 Pro Glu Gly
 20 25
 30

15 Thr Lys Val Thr Val Ala Ala Lys Gln Ala Ala Ala Val
 Val Thr Val
 35 40 45

20 Lys Val Leu Gly Gly Ser Asn Asn Ser Gly Ala Ala Glu
 Ser Met Gly
 50 55 60

25 Thr Ala Arg Arg Val Ala Gln Asp Phe Gln Phe Asp His
 Val Phe Trp
 65 70 75
 80

30 Ser Val Glu Thr Pro Asp Ala Cys Gly Ala Thr Pro Ala
 Thr Gln Ala
 85 90
 95

35 Asp Val Phe Arg Thr Ile Gly Tyr Pro Leu Val Gln His
 Ala Phe Asp
 100 105
 110

Gly Phe Asn Ser Cys Leu Phe Ala Tyr Gly Gln Thr Gly
Ser Gly Lys 115 120 125

5 Thr Tyr Thr Met Met Gly Ala Asp Val Ser Ala Leu Ser
Gly Glu Gly 130 135 140

Asn Gly Val Thr Pro Arg Ile Cys Leu Glu Ile Phe Ala
10 Arg Lys Ala 145 150 155

160

Ser Val Glu Ala Gln Gly His Ser Arg Trp Ile Val Glu
15 Leu Gly Tyr 165 170

175

Val Glu Val Tyr Asn Glu Arg Val Ser Asp Leu Leu Gly
20 Lys Arg Lys 180 185

190

Lys Gly Val Lys Gly Gly Glu Glu Val Tyr Val Asp
25 Val Arg Glu 195 200 205

His Pro Ser Arg Gly Val Phe Leu Glu Gly Gln Arg Leu
Val Glu Val 30 210 215 220

Gly Ser Leu Asp Asp Val Val Arg Leu Ile Glu Ile Gly
Asn Gly Val 225 230 235

35 240

Arg His Thr Ala Ser Thr Lys Met Asn Asp Arg Ser Ser
Arg Ser His
245 250
255
5 Ala Ile Ile Met Leu Leu Leu Arg Glu Glu Arg Thr Met
Thr Thr Lys
260 265
270
10 Ser Gly Glu Thr Ile Arg Thr Ala Gly Lys Ser Ser Arg
Met Asn Leu
275 280 285
15 Val Asp Leu Ala Gly Ser Glu Arg Val Ala Gln Ser Gln
Val Glu Gly
290 295 300
Gln Gln Phe Lys Glu Ala Thr His Ile Asn Leu Ser Leu
20 Thr Thr Leu
305 310 315
320
25 Gly Arg Val Ile Asp Val Leu Ala Asp Met Ala Thr Lys
Gly Ala Lys
325 330
335
30 Ala Gln Tyr Ser Val Ala Pro Phe Arg Asp Ser Lys Leu
Thr Phe Ile
340 345
350
35 Leu Lys Asp Ser Leu Gly Gly Asn Ser Lys Thr Phe Met
Ile Ala Thr
355 360 365

Val Ser Pro Ser Ala Leu Asn Tyr Glu Glu Thr Leu Ser
Thr Leu Arg
370 375 380

5 Tyr Ala Ser Arg Ala Arg Asp Ile Val Asn Val Ala Gln
Val Asn Glu
385 390 395
400

10 Asp Pro Arg Ala Arg Arg Ile Arg Glu Leu Glu Glu Gln
Met Glu Asp
405 410
415

15 Met Arg Gln Ala Met Ala Gly Gly Asp Pro Ala Tyr Val
Ser Glu Leu
420 425
430

20 Lys Lys Lys Leu Ala Leu Leu Glu Ser Glu Ala Gln Lys
Arg Ala Ala
435 440 445

Asp Leu Gln Ala Leu Glu Arg Glu Arg Glu His Asn Gln
25 Val Gln Glu
450 455 460

Arg Leu Leu Arg Ala Thr Glu Ala Glu Lys Ser Glu Leu
Glu Ser Arg
30 465 470 475
480

Ala Ala Ala Leu Gln Glu Glu Met Thr Ala Thr Arg Arg
Gln Ala Asp
35 485 490
495

Lys Met Gln Ala Leu Asn Leu Arg Leu Lys Glu Glu Gln
Ala Arg Lys
500 505
510
5
Glu Arg Glu Leu Leu Lys Glu Met Ala Lys Lys Asp Ala
Ala Leu Ser
515 520 525
10 Lys Val Arg Arg Arg Leu Asp Ala Glu Ile Ala Ser Glu
Arg Glu Lys
530 535 540
Leu Glu Ser Thr Val Ala Gln Leu Glu Arg Glu Gln Arg
15 Glu Arg Glu
545 550 555
560
Val Ala Leu Asp Ala Leu Gln Thr His Gln Arg Lys Leu
20 Gln Glu Ala
565 570
575
Leu Glu Ser Ser Glu Arg Thr Ala Ala Glu Arg Asp Gln
25 Leu Leu Gln
580 585
590
Gln Leu Thr Glu Leu Gln Ser Glu Arg Thr Gln Leu Ser
30 Gln Val Val
595 600 605
Thr Asp Arg Glu Arg Leu Thr Arg Asp Leu Gln Arg Ile
Gln Tyr Glu
35 610 615 620

Tyr Gly Glu Thr Glu Leu Ala Arg Asp Val Ala Leu Cys
Ala Ala Gln
625 630 635
640
5
Glu Met Glu Ala Arg Tyr His Ala Ala Val Phe His Leu
Gln Thr Leu
645 650
655
10 Leu Glu Leu Ala Thr Glu Trp Glu Asp Ala Leu Arg Glu
Arg Ala Leu
660 665
670
15 Ala Glu Arg Asp Glu Ala Ala Ala Ala Glu Leu Asp Ala
Ala Ala Ser
675 680 685
20 Thr Ser Gln Asn Ala Arg Glu Ser Ala Cys Glu Arg Leu
Thr Ser Leu
690 695 700
25 Glu Gln Gln Leu Arg Glu Ser Glu Glu Arg Ala Ala Glu
Leu Ala Ser
705 710 715
720
30 Gln Leu Glu Ala Thr Ala Ala Ala Lys Ser Ser Ala Glu
Gln Asp Arg
725 730
735
35 Glu Asn Thr Arg Ala Thr Leu Glu Gln Gln Leu Arg Glu
Ser Glu Ala
740 745
750

Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Ala Thr Ala
Ala Ala Lys
755 760 765

5 Met Ser Ala Glu Gln Asp Arg Glu Asn Thr Arg Ala Thr
Leu Glu Gln
770 775 780

10 Gln Leu Arg Asp Ser Glu Glu Arg Ala Ala Glu Leu Ala
Ser Gln Leu
785 790 795

800

15 Glu Ser Thr Thr Ala Ala Lys Met Ser Ala Glu Gln Asp
Arg Glu Ser
805 810

815

20 Thr Arg Ala Thr Leu Glu Gln Gln Leu Arg Asp Ser Glu
Glu Arg Ala
820 825

830

25 Ala Glu Leu Ala Ser Gln Leu Glu Ser Thr Thr Ala Ala
Lys Met Ser
835 840 845

30 Ala Glu Gln Asp Arg Glu Ser Thr Arg Ala Thr Leu Glu
Gln Gln Leu
850 855 860

35 Arg Glu Ser Glu Glu Arg Ala Ala Glu Leu Ala Ser Gln
Leu Glu Ser
865 870 875

880

Thr Thr Ala Ala Lys Met Ser Ala Glu Gln Asp Arg Glu
Ser Thr Arg
885 890
895
5 Ala Thr Leu Glu Gln Gln Leu Arg Asp Ser Glu Glu Arg
Ala Ala Glu
900 905
910
10 Leu Ala Ser Gln Leu Glu Ala Thr Ala Ala Ala Lys Ser
Ser Ala Glu
915 920 925
15 Gln Asp Arg Glu Asn Thr Arg Ala Ala Leu Glu Gln Gln
Leu Arg Asp
930 935 940
20 Ser Glu Glu Arg Ala Ala Glu Leu Ala Ser Gln
945 950 955

(2) INFORMATION FOR SEQ ID NO:4:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3319 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA to mRNA

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCTCCACGG CGCTACCCCC TTTCCCGCAT GTGCGACAGT TTCA CGCGTA
CAAACGTCTT 60

	TCTCTCTCCT	TCGCGCGTGT	CGCTATGGGC	GGCGGCGCGT	CGGTGTCTTT
	GATTGCACAG	120			
5	CTCACCGCCT	CGCCATATTT	TCGTCGTGGC	CACGCGACCC	CCCGACCTTC
	CCCTCCTCCG	180			
	CCCCCAAAGA	CAAGCCAGAC	ATACCGACCA	TGCCGTCTGC	CCGCGTCTCT
	GCTTACCAAG	240			
10	CGCGCCACGC	ACCCCTTCCT	CGGCCCTGAA	TCTTCGCGC	GGGCCATAC
	ATTGCATGCA	300			
	CGTCACTACG	CCTGTACACC	TTACACCTCC	TCTTGCCCAC	CCCTTTCCCC
15	TTCTACACGC	360			
	CTAACTACAC	ACACATATAT	ATATATATAT	ATAAAGCGCT	CAACGCACAC
	ATACTGTGGC	420			
20	CAGTATTACT	GCACCAACGT	CTGCCTCTTC	CAGGATGCAC	CCTTCCACTG
	TGCGGCGTGA	480			
	GGCGGAGCGG	GTGAAGGTGT	CGGTGCGCGT	GCGCCCCCTA	AACGAACGTG
	AAAACAATGC	540			
25	CCCGGAAGGG	ACGAAAGTGA	CCGTTGCGGC	GAAACAGGCG	GCCGCCGTGG
	TGACGGTCAA	600			
	GGTCCTGGGA	GGCAGCAACA	ACAGCGGCGC	CGCCGAGTCG	ATGGGGACTG
30	CAAGGCGGGT	660			
	AGCGCAGGAC	TTTCAGTTCG	ACCACGTGTT	CTGGTCTGTG	GAGACGCCGG
	ACGCGTGC GG	720			
35	CGCGACCCCC	GCGACGCAGG	CAGACGTGTT	CCGGACGATC	GGGTACCCGC
	TGGTGCAGCA	780			

	CGCGTTCGAC	GGGTTCAACT	CGTGCTTGT	TGCGTACGGG	CAGACAGGGA
	GCAGGAAGAC	840			
5	GTACACGATG	ATGGGCGCGG	ACGTGAGCGC	GCTTAGTGGT	GAGGGCAACG
	GCCTGACGCC	900			
	GCAGGATCTGC	CTGGAGATCT	TTGCGCGGAA	GGCGAGCGTG	GAGGCGCAGG
	GGCACTCGCG	960			
10	GTGGATCGTG	GAGCTGGGGT	ACGTGGAGGT	GTACAACGAG	CCCGTGTGCG
	ACCTGCTTGG	1020			
	GAAGCGGAAG	AAGGGTGTGA	AGGGCGCGG	CGAGGAGGTG	TACGTGGACG
15	TGCGCGAGCA	1080			
	CCCGAGCCGC	GGCGTGTCC	TGGAGGGGCA	GCGGCTGGTG	GAGGTTGGGA
	GCCTGGACGA	1140			
20	TGTTGTGCGG	CTGATCGAGA	TCGGCAACGG	CGTGCAGCAC	ACCGCTTCGA
	CGAAGATGAA	1200			
	CGACCGGAGC	AGCCGGAGCC	ACGCGATCAT	CATGCTGCTG	CTGCGCGAGG
	AGCGGACGAT	1260			
25	GACGACGAAG	AGCGGGGAGA	CGATCCGTAC	TGCCGGCAAG	AGCAGCCGCA
	TGAACCTTGT	1320			
	GGACCTTGCG	GGGTCTGAGC	GCGTGGCGCA	GTCGCAGGTG	GAGGGGCAGC
	AGTTCAAGGA	1380			
30	GGCGACGCAC	ATCAACCTGT	CGCTGACGAC	GCTCGGGCGC	GTGATCGACG
	TGCTCGCGGA	1440			
	CATGGCGACG	AAGGGTGCAG	AGGCGCAGTA	CAGCGTTGCG	CCGTTCCGCG
35	ACTCGAAGCT	1500			

	GACGTTCATC	CTGAAGGACT	CGCTTGGCGG	GAACTCGAAG	ACGTTCATGA
	TCGCGACTGT	1560			
	GAGCCCGAGC	GCGCTGAACT	ACGAGGAGAC	GCTGAGCACG	CTGCGGTACG
5	CGTCGCGCGC	1620			
	GCGCGACATT	GTGAATGTTG	CGCAGGTGAA	CGAGGACCCG	CGCGCACGGC
	GGATCCCGCA	1680			
10	GCTGGAGGAG	CAGATGGAGG	ACATGCGCA	GGCGATGGCT	GGCGGGGACCC
	CCGCGTACGT	1740			
	GTCTGAGCTG	AAGAAGAAC	TTGCGCTGCT	GGAGTCGGAG	GCGCAGAAC
	GTGCGGCCGA	1800			
15	CCTGCAGGCG	CTGGAGAGGG	AGCGGGAGCA	CAACCAGGTG	CAGGAGCGGC
	TGCTGCGCGC	1860			
	GACGGAGGCG	GAGAAGAGCG	AGCTGGAGTC	GCGTGCAGGCT	GCGCTGCAGG
20	AGGAGATGAC	1920			
	CGCGACTCGA	CGGCAGGCCG	ACAAGATGCA	GGCGCTGAAC	CTGCGGCTGA
	AGGAAGAGCA	1980			
25	GGCGCGCAAG	GAGCGCGAGC	TGCTGAAAGA	GATGGCGAAG	AAGGACGCCG
	CGCTCTCGAA	2040			
	GGTCGGCGA	CGCAAAGACG	CCGAGATAGC	AAGCGAGCGC	GAGAAGCTGG
	AGTCGACCGT	2100			
30	GGCGCAGCTG	GAGCGTGAGC	AGCGCGAGCG	CGAGGTGGCT	CTGGACGCAT
	TGCAGACGCA	2160			
	CCAGAGAAAG	CTGCAGGAAG	CGCTCGAGAG	CTCTGAGCGG	ACAGCCGCCG
35	AAAGGGACCA	2220			

	GCTGCTGCAG	CAGCTAACAG	AGCTTCAGTC	TGAGCGTACG	CAGCTATCAC
	AGGTTGTGAC	2280			
5	CGACCGCGAG	CGGCTTACAC	GCGACTTGCA	GCGTATTCA	TACGAGTACG
	GGGAAACCGA	2340			
	GCTCGCGCGA	GACGTGGCGC	TGTGCGCCGC	GCAGGGAGATG	GAGGCGCGCT
	ACCACGCTGC	2400			
10	TGTGTTCAC	CTGCAAACGC	TCCTGGAGCT	CGCAACCGAG	TGGGAGGACG
	CACTCCCGCA	2460			
	GCGTGCCTT	GCAGAGCGTG	ACGAAGCCGC	TGCAGCCGAA	CTTGATGCCG
15	CAGCCTCTAC	2520			
	TTCCCAAAAC	GCACGTGAAA	GCGCCTGCGA	GCGGCTAAC	AGCCTTGAGC
	AGCAGCTTCG	2580			
20	CGAATCCGAG	GAGCGCGCTG	CGGAGCTGGC	GAGCCAGCTG	GAGGCCACTG
	CTGCTGCGAA	2640			
	GTCGTCGGCG	GAGCAGGACC	GCGAGAACAC	GAGGGCCACG	CTAGAGCAGC
	AGCTTCGCGA	2700			
25	ATCCGAGGCG	CGCGCTGCGG	AGCTGGCGAG	CCAGCTGGAG	GCCACTGCTG
	CTGCGAAGAT	2760			
	GTCAGCGGAG	CAGGACCGCG	AGAACACGAG	GGCCACGCTA	GAGCAGCAGC
30	TTCGTGACTC	2820			
	CGAGGGAGCGC	GCTGCGGAGC	TGGCGAGCCA	GCTGGAGTCC	ACTACTGCTG
	CGAAGATGTC	2880			
	AGCGGAGCAG	GACCGCGAGA	GCACGAGGGC	CACGCTAGAG	CAGCAGCTTC
35	GTGACTCCGA	2940			

	GGAGCGCGCT	GCGGAGCTGG	CGAGCCAGCT	GGAGTCCACT	ACTGCTGCGA
	AGATGTCAGC	3000			
	GGAGCAGGAC	CGCGAGAGCA	CGAGGGCCAC	GCTAGAGCAG	CAGCTTCGCG
5	AATCCGAGGA	3060			
	GCGCGCTGCG	GAGCTGGCGA	GCCAGCTGGA	GTCCACTACT	GCTGCGAAGA
	TGTCAGCGGA	3120			
10	GCAGGACCAGC	GAGAGCACGA	GGGCCACGCT	AGAGCAGCAG	CTTCGTGACT
	CCGAGGAGCG	3180			
	CGCTGCGGAG	CTGGCGAGCC	AGCTGGAGGC	CACTGCTGCT	GCAGAACGCGT
	CGGCGGAGCA	3240			
15	GGACCGCGAG	AACACGAGGG	CCGCGTTGGA	GCAGCAGCTT	CGTGACTCCG
	AGGAGCGCGC	3300			
	CGCGGAGCTG				GCGAGCCAG
20	3319				

(2) INFORMATION FOR SEQ ID NO:5:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 39 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

35 Leu Glu Gln Gln Leu Arg (Asp/Glu) Ser Glu (Glu/Ala)
 Arg Ala Ala

1

5

10

Glu Leu Ala Ser Gln Leu Glu (Ala/Ser) Thr (Ala/Thr)
Ala Ala Lys
15 20
5 25

(Met/Ser) Ser Ala Glu Gln Asp Arg Glu (Asn/Ser) Thr
Arg Ala
10 30 35
(Thr/Ala)

Claims

1. A method for detecting asymptomatic or sub-clinical *Leishmania* infection in a biological sample, comprising:

(a) contacting a biological sample with a polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(b) detecting in the biological sample the presence of antibodies that bind to the polypeptide, thereby detecting asymptomatic or sub-clinical *Leishmania* infection in the biological sample.

2. The method of claim 1 wherein the epitope of LcP0 comprises amino acids 306-322 of SEQ ID NO:2, or a variant thereof that differs only in conservative substitutions and/or modifications.

3. The method of claim 1 wherein the polypeptide is bound to a solid support.

4. The method of claim 3 wherein the solid support comprises nitrocellulose, latex or a plastic material.

5. The method of claim 3 wherein the step of detecting comprises:

(a) removing unbound sample from the solid support;

(b) adding a detection reagent to the solid support; and

(c) determining the level of detection reagent bound to the solid support, relative to a predetermined cutoff value, thereby detecting asymptomatic or sub-clinical *Leishmania* infection in the biological sample.

6. A method for detecting *Leishmania* infection in a biological sample, comprising:

(a) contacting a biological sample with a first amino acid sequence comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications;

(b) contacting the biological sample with a second amino acid sequence comprising Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gin Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21

is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(c) detecting in the sample the presence of antibodies that bind to one or both of the amino acid sequences, thereby detecting *Leishmania* infection in the biological sample.

7. The method of claim 6 wherein the epitope of LcP0 comprises amino acids 306-322 of SEQ ID NO:2, or a variant thereof that differs only in conservative substitutions and/or modifications.

8. The method of claim 6 wherein the first and second amino acid sequences are bound to a solid support.

9. The method of claim 8 wherein the solid support comprises nitrocellulose, latex or a plastic material.

10. The method of claim 8 wherein the step of detecting comprises:

- (a) removing unbound sample from the solid support;
- (b) adding a detection reagent to the solid support; and
- (c) determining the level of detection reagent bound to the solid support, relative to a predetermined cutoff value, therefrom detecting *Leishmania* in the biological sample.

11. A method for identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis, comprising:

(a) contacting a biological sample obtained from a patient afflicted with asymptomatic or subclinical leishmaniasis with a polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(b) detecting in the sample the presence of antibodies that bind to the polypeptide, thereby identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis.

12. The method of claim 11 wherein the polypeptide is bound to a solid support.

13. The method of claim 12 wherein the solid support comprises nitrocellulose, latex or a plastic material.

14. The method of claim 12 wherein the step of detecting comprises:

- (a) removing unbound sample from the solid support;
- (b) adding a detection reagent to the solid support; and
- (c) determining the level of detection reagent bound to the solid support, relative to a predetermined cutoff value, therefrom identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis.

15. A method for identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis, comprising:

(a) contacting a biological sample obtained from a patient afflicted with asymptomatic or subclinical leishmaniasis with a first polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications;

(b) independently contacting the biological sample with a second polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and

(c) detecting in the sample the presence of antibodies that bind to the first and/or second polypeptides, thereby identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis.

16. The method of claim 15 wherein the epitope of LcP0 comprises amino acids 306-322 of SEQ ID NO:2, or a variant thereof that differs only in conservative substitutions and/or modifications.

17. The method of claim 15 wherein the first and second polypeptides are each bound to a separate solid support.

18. The method of claim 17 wherein the solid supports comprise nitrocellulose, latex or a plastic material.

19. The method of claim 17 wherein the step of detecting comprises:

- (a) removing unbound sample from each solid support;
- (b) adding a detection reagent to each solid support; and
- (c) comparing the level of detection reagent bound to each solid support, relative to a predetermined cutoff value, therefrom identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis.

20. The method of any of claims 1, 6, 11 or 15 wherein the biological sample is selected from the group consisting of blood, serum, plasma, saliva, cerebrospinal fluid and urine.

21. The method of claim 20 wherein the biological sample is whole blood or plasma.

22. The method of any of claims 5, 10, 14 or 19 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

23. The method of claim 22 wherein the binding agent is selected from the group consisting of anti-immunoglobulin, Protein G, Protein A and lectins.

24. The method of claim 22 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

25. A polypeptide comprising amino acids 306-322 of SEQ ID NO:2.

26. A diagnostic kit for detecting asymptomatic or sub-clinical leishmaniasis in a biological sample, comprising:

- (a) a polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; and
- (b) a detection reagent.

27. A diagnostic kit for detecting *Leishmania* infection in a biological sample, comprising:

- (a) a first amino acid sequence comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications;
- (b) a second amino acid sequence comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and
- (c) a detection reagent.

28. A diagnostic kit for identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis, comprising:

- (a) a polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and
- (b) a detection reagent.

29. A diagnostic kit for identifying a patient afflicted with asymptomatic or subclinical leishmaniasis that is likely to develop acute visceral leishmaniasis, comprising:

- (a) a first polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications;
- (b) a second polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications; and
- (c) a detection reagent.

30. The kit of any of claims 26-29 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

31. The kit of claim 30 wherein the binding agent is selected from the group consisting of anti-immunoglobulin, Protein G, Protein A and lectins.

32. The kit of claim 30 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

33. A pharmaceutical composition comprising:

- (a) a polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; and
- (b) a physiologically acceptable carrier.

34. The pharmaceutical composition of claim 33, further comprising a second polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications.

35. The pharmaceutical composition of claim 33, wherein the polypeptide further comprises the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications.

36. A vaccine for stimulating the production of antibodies that bind to *Leishmania*, comprising:

- (a) a polypeptide comprising an epitope of LcP0, or a variant thereof that differs only in conservative substitutions and/or modifications; and
- (b) an adjuvant.

37. The vaccine of claim 36, further comprising a second polypeptide comprising the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications.

38. The vaccine of claim 36, wherein the polypeptide further comprises the amino acid sequence Leu Glu Gln Gln Leu Arg Xaa Ser Glu Xaa Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Xaa Thr Xaa Ala Ala Lys Xaa Ser Ala Glu Gln Asp Arg Glu Xaa Thr Arg Ala Xaa, wherein Xaa at position 7 is Asp or Glu, at position 10 is Glu or Ala, at position 21 is Ala or Ser, at position 23 is Ala or Thr, at position 27 is Met or Ser, at position 35 is Asn or Ser, and at position 39 is Thr or Ala, or a variant thereof that differs only in conservative substitutions and/or modifications.

39. An isolated DNA sequence encoding the polypeptide of claim 25.

40. A recombinant expression vector comprising the isolated DNA sequence of claim 39.

41. A host cell transformed with the expression vector of claim 40.

42. The host cell of claim 41 wherein the host cell is selected from the group consisting of *E. coli*, yeast, insect cell lines and mammalian cell lines.

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ATAGCCAAGG CTATTGCAAG TCTCACAAAG ATG CCG TCT ATC ACC ACT GCC AAG Met Pro Ser Ile Thr Thr Ala Lys	53
1 5	
CGC GAG TAC GAG GAG CGC CTC GTC GAC TGC CTG ACC AAG TAC AGC TGC Arg Glu Tyr Glu Glu Arg Leu Val Asp Cys Leu Thr Lys Tyr Ser Cys	101
10 15 20	
GTC CTG TTC GTG GGC ATG GAC AAC GTC CGC TCG CAG CAG GTG CAC GAT Val Leu Phe Val Gly Met Asp Asn Val Arg Ser Gln Gln Val His Asp	149
25 30 35 40	
GTC CGC CGT GGC TGT CGC GGC AAG GCC GAG TTC ATT ATG GGC AAG AAG Val Arg Arg Gly Cys Arg Gly Lys Ala Glu Phe Ile Met Gly Lys Lys	197
45 50 55	
ACG CTG CAG GCG AAG ATC GTG GAG AAG CGC GCG CAA GCC AAG GAC GCG Thr Leu Gln Ala Lys Ile Val Glu Lys Arg Ala Gln Ala Lys Asp Ala	245
60 65 70	
AGC CCC GAG GCG AAG CCT TTC AAC GAT CAG TGT GAG GAG TAC AAC CTG Ser Pro Glu Ala Lys Pro Phe Asn Asp Gln Cys Glu Glu Tyr Asn Leu	293
75 80 85	
CTG AGC GGC AAC ACC GGC CTC ATC TTC ACT AAC AAC GCT GTC CAG GAG Leu Ser Gly Asn Thr Gly Leu Ile Phe Thr Asn Asn Ala Val Gln Glu	341
90 95 100	
ATC ACC TCT GTG CTT GAC GGC CAC CGC GTG AAG GCC CCG GCG CGT GTC Ile Thr Ser Val Leu Asp Gly His Arg Val Lys Ala Pro Ala Arg Val	389
105 110 115 120	
GGA GCG ATT CCG TGC GAC GTG GTT GTG CCT GCT GGC AGC ACC GGC ATG Gly Ala Ile Pro Cys Asp Val Val Val Pro Ala Gly Ser Thr Gly Met	437
125 130 135	
GAG CCG ACC CAG ACG TCC TTC CAG GCG CTG AAC ATT GCG ACG AAG Glu Pro Thr Gln Thr Ser Phe Phe Gln Ala Leu Asn Ile Ala Thr Lys	485
140 145 150	
ATT GCC AAG GGT ATG GTG GAG ATC GTG ACG GAG AAG AAG GTG CTG AGC Ile Ala Lys Gly Met Val Glu Ile Val Thr Glu Lys Lys Val Leu Ser	533
155 160 165	
GTC GGC GAC AAG GTG GAC AAC TCG ACG GCG ACG CTG CTG CAA AAG CTG Val Gly Asp Lys Val Asp Asn Ser Thr Ala Thr Leu Leu Gln Lys Leu	581
170 175 180	
AAC ATC AGC CCG TTC TAC TAC CAG GTG AAT GTG CTG TCC GTG TGG GAC Asn Ile Ser Pro Phe Tyr Tyr Gln Val Asn Val Leu Ser Val Trp Asp	629
185 190 195 200	
CGC GGT GTG CTG TTC ACC CGC GAG GAC CTC ATG ATG ACG GAG GAC ATG Arg Gly Val Leu Phe Thr Arg Glu Asp Leu Met Met Thr Glu Asp Met	677
205 210 215	
GTG GAG AAG ATG CTG ATG GAA GGC CTG AGC AAC GTT GCG GCG ATG GCG Val Glu Lys Met Leu Met Glu Gly Leu Ser Asn Val Ala Ala Met Ala	725
220 225 230	
CTG GGT GCT GGC ATC CCG ACG TCT TCG ACG ATT GGC CCG ATG CTG GTG Leu Gly Ala Gly Ile Pro Thr Ser Ser Thr Ile Gly Pro Met Leu Val	773
235 240 245	

Fig. 1A

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GAC	GCC	TTC	AAG	AAC	CTG	CTG	GCT	GTC	TCC	GTG	GCG	ACC	TCG	TAC	GAG		821
Asp	Ala	Phe	Lys	Asn	Leu	Leu	Ala	Val	Ser	Val	Ala	Thr	Ser	Tyr	Glu		
250					255					260							
TTC	GAG	GAG	CAC	AAC	GGC	AAG	GAG	CTG	CGC	GAG	GCC	GCG	ATC	AAC	GGC		869
Phe	Glu	Glu	His	Asn	Gly	Lys	Glu	Leu	Arg	Glu	Ala	Ala	Ile	Asn	Gly		
265					270					275							
CTG	CTG	GCC	GGC	TCT	GGC	TCG	GCT	GCT	GCG	GAG	CCC	GCC	GCT	GCC	GCG		917
Leu	Leu	Ala	Gly	Ser	Gly	Ser	Ala	Ala	Ala	Glu	Pro	Ala	Ala	Ala	Ala		
285					290					295							
CCG	GCC	GCC	CCT	AGC	GCT	GCT	GCC	AAG	GAG	GAG	CCG	GAG	GAG	AGC	GAC		965
Pro	Ala	Ala	Pro	Ser	Ala	Ala	Ala	Lys	Glu	Glu	Pro	Glu	Glu	Ser	Asp		
300					305					310							
GAG	GAC	GAC	TTC	GGC	ATG	GGC	GGT	CTC	TTC	TAA	GCGACTCGCT	ATCCGCCACC				1018	
Glu	Asp	Asp	Phe	Gly	Met	Gly	Gly	Leu	Phe	*							
315					320												
CAGCACCGTC	GAGTGTCGT	GCCTCGCAT	GGTGGACAGT	GGCGAGCGTG	TGATGCCCTT											1078	
GGATCATCAG	GAAGCAACTC	TCTCCCTTTC	TCTGGGTGTT	CTTCGTTCT	TCTTCATT											1138	
GTTTTGATC	GCCGTGGCGC	TGCGGCGATC	GCTCAGTTCT	TATTTCGAT	CAACCAACAA											1198	
CGAA																1202	

Fig. 1B

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Met His Pro Ser Thr Val Arg Arg Glu Ala Glu Arg Val Lys Val Ser
 1 5 10 15
 Val Arg Val Arg Pro Leu Asn Glu Arg Glu Asn Asn Ala Pro Glu Gly
 20 25 30
 Thr Lys Val Thr Val Ala Ala Lys Gln Ala Ala Ala Val Val Thr Val
 35 40 45
 Lys Val Leu Gly Gly Ser Asn Asn Ser Gly Ala Ala Glu Ser Met Gly
 50 55 60
 Thr Ala Arg Arg Val Ala Gln Asp Phe Gln Phe Asp His Val Phe Trp
 65 70 75 80
 Ser Val Glu Thr Pro Asp Ala Cys Gly Ala Thr Pro Ala Thr Gln Ala
 85 90 95
 Asp Val Phe Arg Thr Ile Gly Tyr Pro Leu Val Gln His Ala Phe Asp
 100 105 110
 Gly Phe Asn Ser Cys Leu Phe Ala Tyr Gly Gln Thr Gly Ser Gly Lys
 115 120 125
 Thr Tyr Thr Met Met Gly Ala Asp Val Ser Ala Leu Ser Gly Glu Gly
 130 135 140
 Asn Gly Val Thr Pro Arg Ile Cys Leu Glu Ile Phe Ala Arg Lys Ala
 145 150 155 160
 Ser Val Glu Ala Gln Gly His Ser Arg Trp Ile Val Glu Leu Gly Tyr
 165 170 175
 Val Glu Val Tyr Asn Glu Arg Val Ser Asp Leu Leu Gly Lys Arg Lys
 180 185 190
 Lys Gly Val Lys Gly Gly Glu Glu Val Tyr Val Asp Val Arg Glu
 195 200 205
 His Pro Ser Arg Gly Val Phe Leu Glu Gly Gln Arg Leu Val Glu Val
 210 215 220
 Gly Ser Leu Asp Asp Val Val Arg Leu Ile Glu Ile Gly Asn Gly Val
 225 230 235 240
 Arg His Thr Ala Ser Thr Lys Met Asn Asp Arg Ser Ser Arg Ser His
 245 250 255
 Ala Ile Ile Met Leu Leu Leu Arg Glu Glu Arg Thr Met Thr Thr Lys
 260 265 270
 Ser Gly Glu Thr Ile Arg Thr Ala Gly Lys Ser Ser Arg Met Asn Leu
 275 280 285
 Val Asp Leu Ala Gly Ser Glu Arg Val Ala Gln Ser Gln Val Glu Gly
 290 295 300
 Gln Gln Phe Lys Glu Ala Thr His Ile Asn Leu Ser Leu Thr Thr Leu
 305 310 315 320
 Gly Arg Val Ile Asp Val Leu Ala Asp Met Ala Thr Lys Gly Ala Lys
 325 330 335

Fig. 2A

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Ala Gin Tyr Ser Val Ala Pro Phe Arg Asp Ser Lys Leu Thr Phe Ile
 340 345 350
 Leu Lys Asp Ser Leu Gly Gly Asn Ser Lys Thr Phe Met Ile Ala Thr
 355 360 365
 Val Ser Pro Ser Ala Leu Asn Tyr Glu Glu Thr Leu Ser Thr Leu Arg
 370 375 380
 Tyr Ala Ser Arg Ala Arg Asp Ile Val Asn Val Ala Gln Val Asn Glu
 385 390 395 400
 Asp Pro Arg Ala Arg Arg Ile Arg Glu Leu Glu Glu Gln Met Glu Asp
 405 410 415
 Met Arg Gln Ala Met Ala Gly Gly Asp Pro Ala Tyr Val Ser Glu Leu
 420 425 430
 Lys Lys Leu Ala Leu Leu Glu Ser Glu Ala Gln Lys Arg Ala Ala
 435 440 445
 Asp Leu Gln Ala Leu Glu Arg Glu Arg Glu His Asn Gln Val Gln Glu
 450 455 460
 Arg Leu Leu Arg Ala Thr Glu Ala Glu Lys Ser Glu Leu Glu Ser Arg
 465 470 475 480
 Ala Ala Ala Leu Gln Glu Glu Met Thr Ala Thr Arg Arg Gln Ala Asp
 485 490 495
 Lys Met Gln Ala Leu Asn Leu Arg Leu Lys Glu Glu Gln Ala Arg Lys
 500 505 510
 Glu Arg Glu Leu Leu Lys Glu Met Ala Lys Lys Asp Ala Ala Leu Ser
 515 520 525
 Lys Val Arg Arg Arg Leu Asp Ala Glu Ile Ala Ser Glu Arg Glu Lys
 530 535 540
 Leu Glu Ser Thr Val Ala Gln Leu Glu Arg Glu Gln Arg Glu Arg Glu
 545 550 555 560
 Val Ala Leu Asp Ala Leu Gln Thr His Gln Arg Lys Leu Gln Glu Ala
 565 570 575
 Leu Glu Ser Ser Glu Arg Thr Ala Ala Glu Arg Asp Gln Leu Leu Gln
 580 585 590
 Gln Leu Thr Glu Leu Gln Ser Glu Arg Thr Gln Leu Ser Gln Val Val
 595 600 605
 Thr Asp Arg Glu Arg Leu Thr Arg Asp Leu Gln Arg Ile Gln Tyr Glu
 610 615 620
 Tyr Gly Glu Thr Glu Leu Ala Arg Asp Val Ala Leu Cys Ala Ala Gln
 625 630 635 640
 Glu Met Glu Ala Arg Tyr His Ala Ala Val Phe His Leu Gln Thr Leu
 645 650 655
 Leu Glu Leu Ala Thr Glu Trp Glu Asp Ala Leu Arg Glu Arg Ala Leu
 660 665 670
 Ala Glu Arg Asp Glu Ala Ala Ala Glu Leu Asp Ala Ala Ala Ser
 675 680 685

Fig. 2B

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Thr Ser Gln Asn Ala Arg Glu Ser Ala Cys Glu Arg Leu Thr Ser Leu
 690 695 700
 Glu Gln Gln Leu Arg Glu Ser Glu Glu Arg Ala Ala Glu Leu Ala Ser
 705 710 715 720
 Gln Leu Glu Ala Thr Ala Ala Ala Lys Ser Ser Ala Glu Gln Asp Arg
 725 730 735
 Glu Asn Thr Arg Ala Thr Leu Glu Gln Gln Leu Arg Glu Ser Glu Ala
 740 745 750
 Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Ala Thr Ala Ala Ala Lys
 755 760 765
 Met Ser Ala Glu Gln Asp Arg Glu Asn Thr Arg Ala Thr Leu Glu Gln
 770 775 780
 Gln Leu Arg Asp Ser Glu Glu Arg Ala Ala Glu Leu Ala Ser Gln Leu
 785 790 795 800
 Glu Ser Thr Thr Ala Ala Lys Met Ser Ala Glu Gln Asp Arg Glu Ser
 805 810 815
 Thr Arg Ala Thr Leu Glu Gln Gln Leu Arg Asp Ser Glu Glu Arg Ala
 820 825 830
 Ala Glu Leu Ala Ser Gln Leu Glu Ser Thr Thr Ala Ala Lys Met Ser
 835 840 845
 Ala Glu Gln Asp Arg Glu Ser Thr Arg Ala Thr Leu Glu Gln Gln Leu
 850 855 860
 Arg Glu Ser Glu Glu Arg Ala Ala Glu Leu Ala Ser Gln Leu Glu Ser
 865 870 875 880
 Thr Thr Ala Ala Lys Met Ser Ala Glu Gln Asp Arg Glu Ser Thr Arg
 885 890 895
 Ala Thr Leu Glu Gln Gln Leu Arg Asp Ser Glu Glu Arg Ala Ala Glu
 900 905 910
 Leu Ala Ser Gln Leu Glu Ala Thr Ala Ala Ala Lys Ser Ser Ala Glu
 915 920 925
 Gln Asp Arg Glu Asn Thr Arg Ala Ala Leu Glu Gln Gln Leu Arg Asp
 930 935 940
 Ser Glu Glu Arg Ala Ala Glu Leu Ala Ser Gln
 945 950 955

Fig. 2C

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GCTCCCACGG CGCTACCCCC	TTTCCCGCAT GTGCGACAGT	TTCACCGCGTA CAAACGTCTT	60
TCTCTCTCCT TCGCGCGTGT	CGCTATGGGC GGCAGGCGCGT	CGGTGTCTTT GATTGCACAG	120
CTCACCGCCT CGCCATATT	TCGTCGTGGC CACCGGACCC	CCCGACCTTC CCCTCCTCCG	180
CCCCCAAAGA CAAGCCAGAC	ATACCGACCA TGCCGTCTGC	CCGGCGTCTCT GCTTACCAAG	240
CGCGGCCACGC ACCCCCTCCT	CGGCCCTGAA TCTTCGCGC	GGCGCCATAC ATTGCATGCA	300
CGTCACTACG CCTGTACACC	TTACACCTCC TCTTGCCCAC	CCCTTCCCCC TTCTACACGC	360
CTAACTACAC ACACATATAT	ATATATATAT ATAAAGCGCT	CAACGCACAC ATACTGTGGC	420
CAGTATTACT GCACCAACGT	CTGCCTCTTC CAGGATGCAC	CCTTCCACTG TGCGGCGTGA	480
GGCGGAGCGG GTGAAGGTGT	CGGTGCGCGT GCGCCCCCTA	AACGAACGTG AAAACAATGC	540
CCCGGAAGGG ACGAAAGTGA	CCGTTGCGGC GAAACAGGCG	GCCGCCGTGG TGACGGTCAA	600
GGTCCTGGGA GGCAGCAACA	ACAGCGGCCG CGCCGAGTCG	ATGGGGACTG CAAGGGCGGGT	660
AGCGCAGGAC TTTCAGTTCG	ACCACGTGTT CTGGTCTGTG	GAGACGCCGG ACGCGTGCAGG	720
CGCGACCCCC GCGACCGAGG	CAGACGTGTT CCGGACGATC	GGGTACCCGC TGGTGCAGCA	780
CGCGTTGAC GGGTTCAACT	CGTGCTTGTT TCGGTACCGG	CAGACAGGGA GCGGGAAAGAC	840
GTACACGATG ATGGGCGCGG	ACGTGAGCGC GCTTAGTGGT	GAGGGCAACG GCGTGACGCC	900
CGGGATCTGC CTGGAGATCT	TTGCGCGGAA GGCAGCGTG	GAGGCCGAGG GGCACACTCGCG	960
GTGGATCGTG GAGCTGGGT	ACGTGGAGGT GTACACGAG	CGCGTGTGCG ACCTGTTGG	1020
GAAGCGGAAG AAGGGTGTGA	AGGGCGGCAG CGAGGAGGTG	TACGTGGACG TGCGCGAGCA	1080
CCCGAGCCGC GCGGTGTTCC	TGGAGGGCA GCGGCTGGTG	GAGGTTGGGA GCCTGGACGA	1140
TGTTGTGCGG CTGATCGAGA	TCGGCAACGG CGTGCAGGCAC	ACCGCTTCGA CGAAGATGAA	1200
CGACCGGAGC AGCCGGAGCC	ACCGATCAT CATGCTGCTG	CTGCCGGAGG AGCGGACCAT	1260
GACGACGAAG AGCGGGGAGA	CGATCCGTAC TGCCGGCAAG	AGCAGCCGCA TGAACCTTGT	1320
GGACCTTGCAG GGGTCTGAGC	GCGTGGCGCA GTCGCAGGTG	GAGGGGCAGC AGTTCAAGGA	1380
GGCGACGCAC ATCAACCTGT	CGCTGACGAC GCTCGGGCGC	GTGATCGACG TGCTCGCGGA	1440
CATGGCGACG AAGGGTGCAGA	AGGCGCAGTA CAGCGTTGCG	CCGTTCCGCG ACTCGAAGCT	1500
GACGTTCATC CTGAAGGACT	CGCTTGGCGG GAACTCGAAG	ACGTTCATGA TCGCGACTGT	1560
GAGCCCGAGC GCGCTGAAC	ACGAGGAGAC GCTGAGCACG	CTGCGGTACG CGTCGCGCGC	1620
GCGCGACATT GTGAATGTG	CGCAGGTGAA CGAGGACCCG	CGCGCACCGC GGATCCGCGA	1680
GCTGGAGGAG CAGATGGAGG	ACATGCGGCA GGCAGATGGCT	GGCGGGCGACC CCCGCGTACGT	1740
GTCTGAGCTG AAGAAGAAGC	TTGCGCTGCT GGAGTCGGAG	GCGCAGAAGC GTGCGGCGGA	1800
CCTGCAGGCG CTGGAGAGGG	AGCGGGAGCA CAACCAGGTG	CAGGAGCGGC TGCTGCGCGC	1860
GACGGAGGCG GAGAAGAGCG	AGCTGGAGTC GCGCTGCAGG	AGGAGATGAC	1920

Fig. 3A

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CGCGACTCGA CGGCAGGCCG ACAAGATGCA	GGCGCTGAAC CTGGGGCTGA AGGAAGAGCA	1980
GGCGCGCAAG GAGCGCGAGC TGCTGAAAGA	GATGGCGAAG AAGGACGCCG CGCTCTCGAA	2040
GGTTCGGCGA CGCAAAGACG CCGAGATAGC	AAGCGAGCGC GAGAAGCTGG AGTCGACCGT	2100
GGCGCAGCTG GAGCGTGAGC AGCGCGAGCG	CGAGGTGGCT CTGGACGCAT TGCAGACGCA	2160
CCAGAGAAAG CTGCAGGAAG CGCTCGAGAG	CTCTGAGCGG ACAGCCGCGG AAAGGGACCA	2220
GCTGCTGCAG CAGCTAACAG AGCTTCAGTC	TGAGCGTACG CAGCTATCAC AGGTTGTGAC	2280
CGACCGCGAG CGGCTTACAC GCGACTTGCA	GCCTATTCAAG TACGAGTACG GGGAAACCGA	2340
GCTCGCGCGA GACGTGGCGC	TGTGCGCCGC GCAGGAGATG GAGGCGCGCT ACCACGCTGC	2400
TGTGTTTAC CTGCAAACGC TCCTGGAGCT	CGCAACCGAG TGGGAGGACG CACTCCGCGA	2460
GCCTGCGCTT GCAGAGCGTG ACGAAGCCGC	TGCAAGCCGAA CTTGATGCCG CAGCCTCTAC	2520
TTCCCAAAAC GCACGTGAAA GCGCCTGCGA	GCCTGTAACC AGCCTTGAGC AGCAGCTTCG	2580
CGAATCCGAG GAGCGCGCTG CGGAGCTGGC	GAGCCAGCTG GAGGCCACTG CTGCTGCGAA	2640
GTCGTCGGCG GAGCAGGACC GCGAGAACAC	GAGGGCCACG CTAGAGCAGC AGCTTCGCGA	2700
ATCCGAGGCG CGCGCTGCGG AGCTGGCGAG	CCAGCTGGAG GCCACTGCTG CTGCGAAGAT	2760
GTCAGCGGAG CAGGACCGCG AGAACACGAG	GGCCACGCTA GAGCAGCAGC TTCGTGACTC	2820
CGAGGAGCGC GCTGCGGAGC TGGCGAGCCA	GCTGGAGTCC ACTACTGCTG CGAAGATGTC	2880
AGCGGAGCAG GACCGCGAGA GCACGAGGGC	CACGCTAGAG CAGCAGCTTC GTGACTCCGA	2940
GGAGCGCGCT CGGGAGCTGG CGAGCCAGCT	GGAGTCCACT ACTGCTGCGA AGATGTCAGC	3000
GGAGCAGGAC CGCGAGAGCA CGAGGGCCAC	GCTAGAGCAG CAGCTTCGCG AATCCGAGGA	3060
GCGCGCTGCG GAGCTGGCGA GCCAGCTGGA	GTCCACTACT GCTGCGAAGA TGTCAAGCGGA	3120
GCAGGACCGC GAGAGCACGA GGGCCACGCT	AGAGCAGCAG CTTCGTGACT CCGAGGAGCG	3180
CGCTGCGGAG CTGGCGAGCC AGCTGGAGGC	CACTGCTGCT GCGAAGTCGT CGGCGGAGCA	3240
GGACCGCGAG AACACGAGGG CCCCGTTGGA	GCAGCAGCTT CGTGAUTCCG AGGAGCGCGC	3300
CGCGGAGCTG GCGAGCCAG		3319

Fig. 3B

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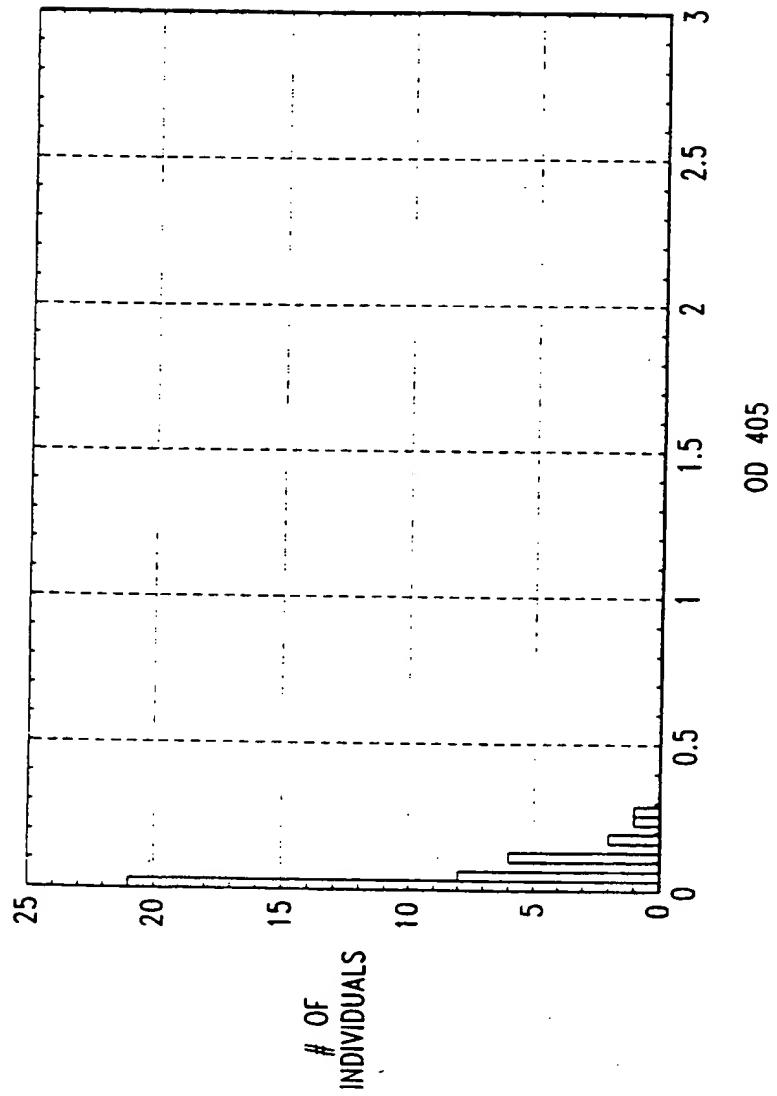


Fig. 4A

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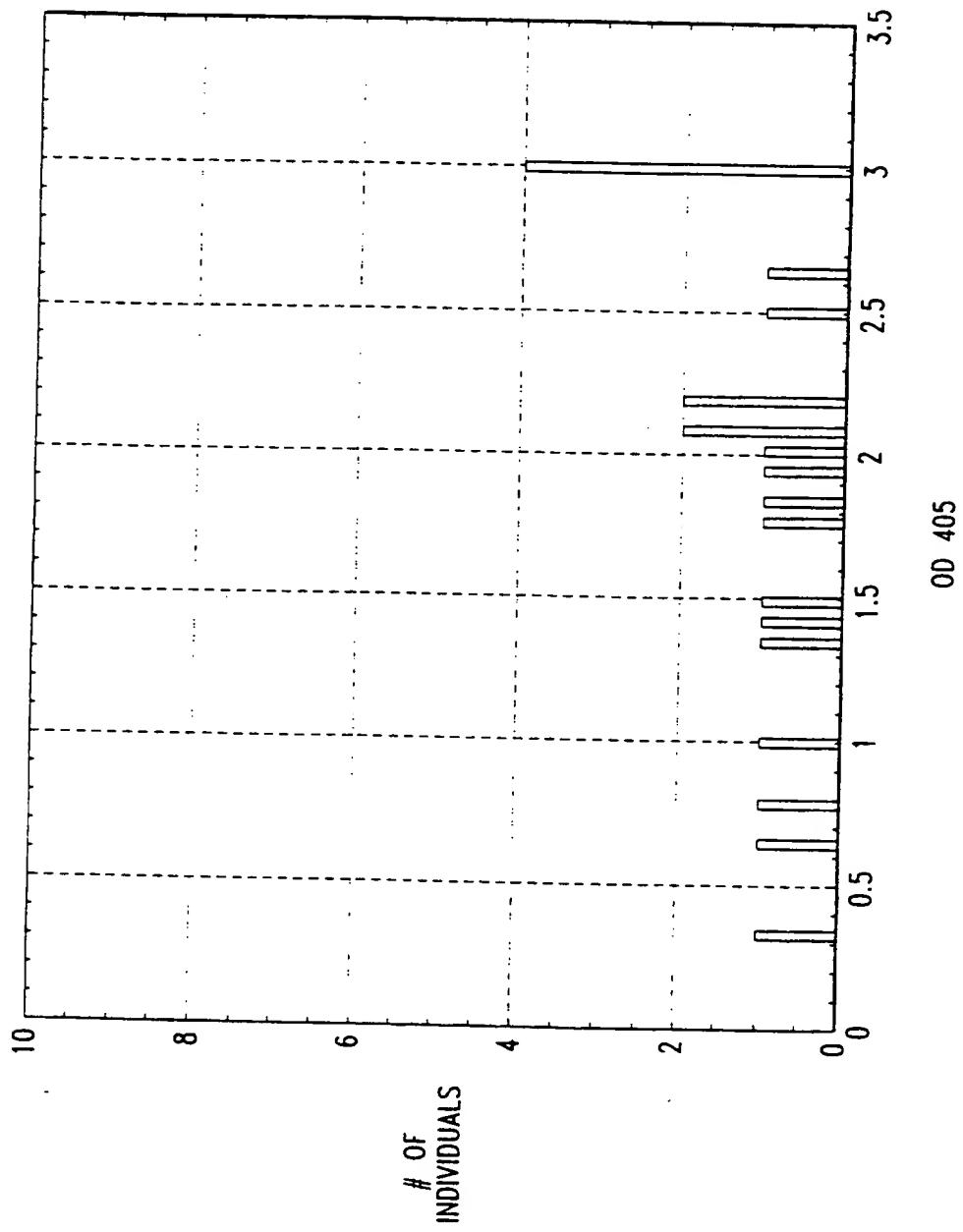


Fig. 4B

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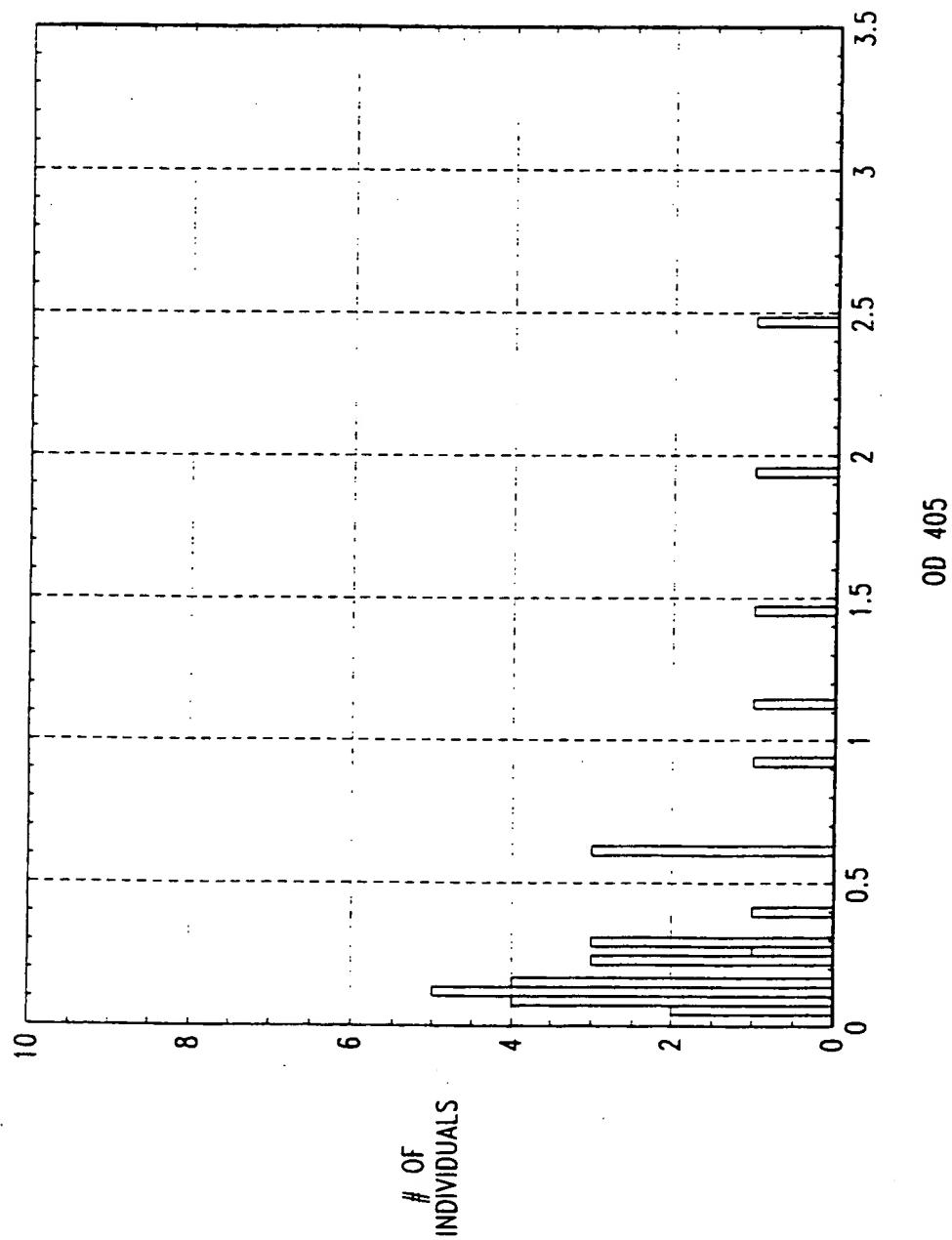


Fig. 4C

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□ rLcb1
■ LcPopep



Fig. 5A

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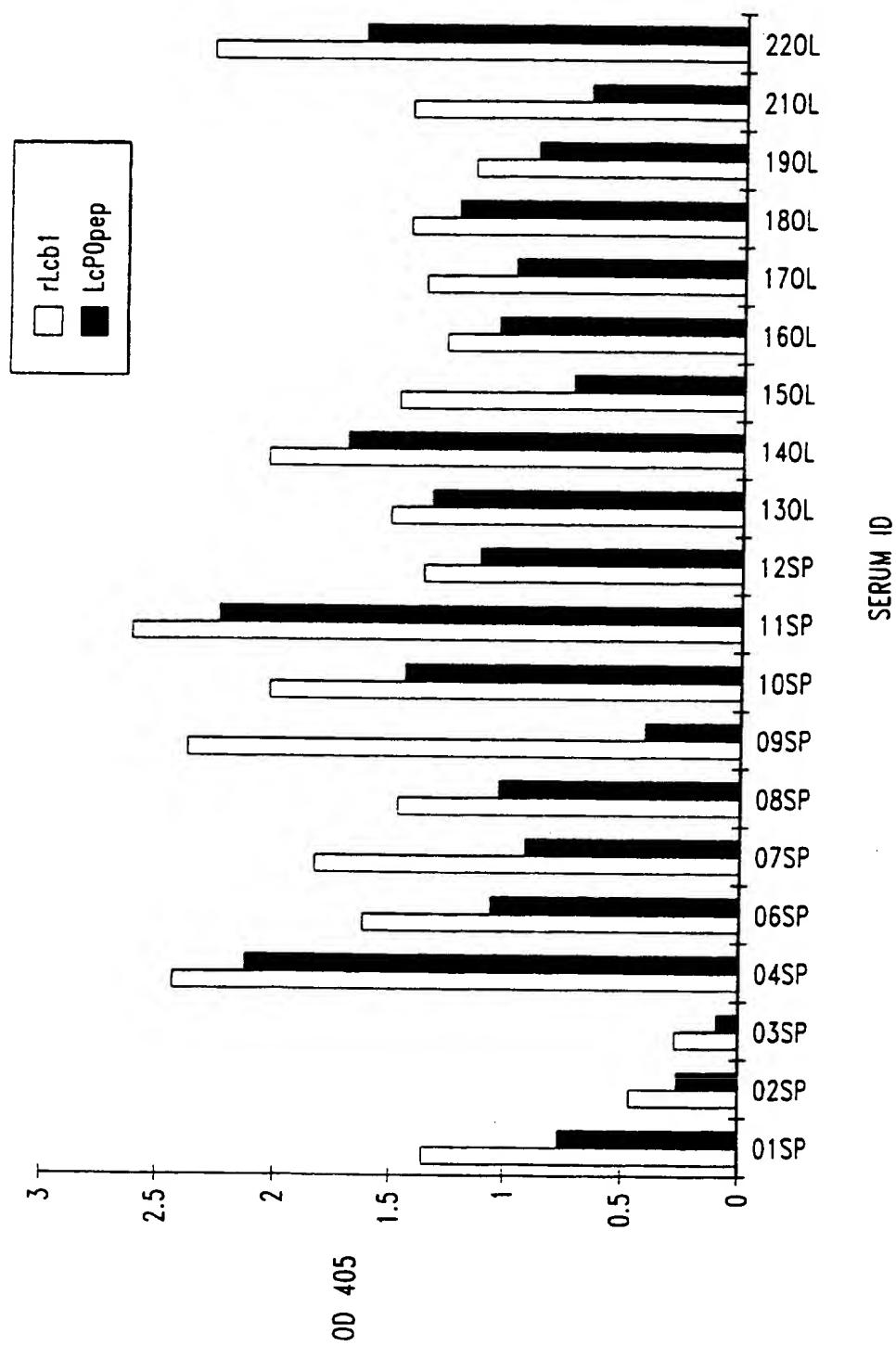


Fig. 5B

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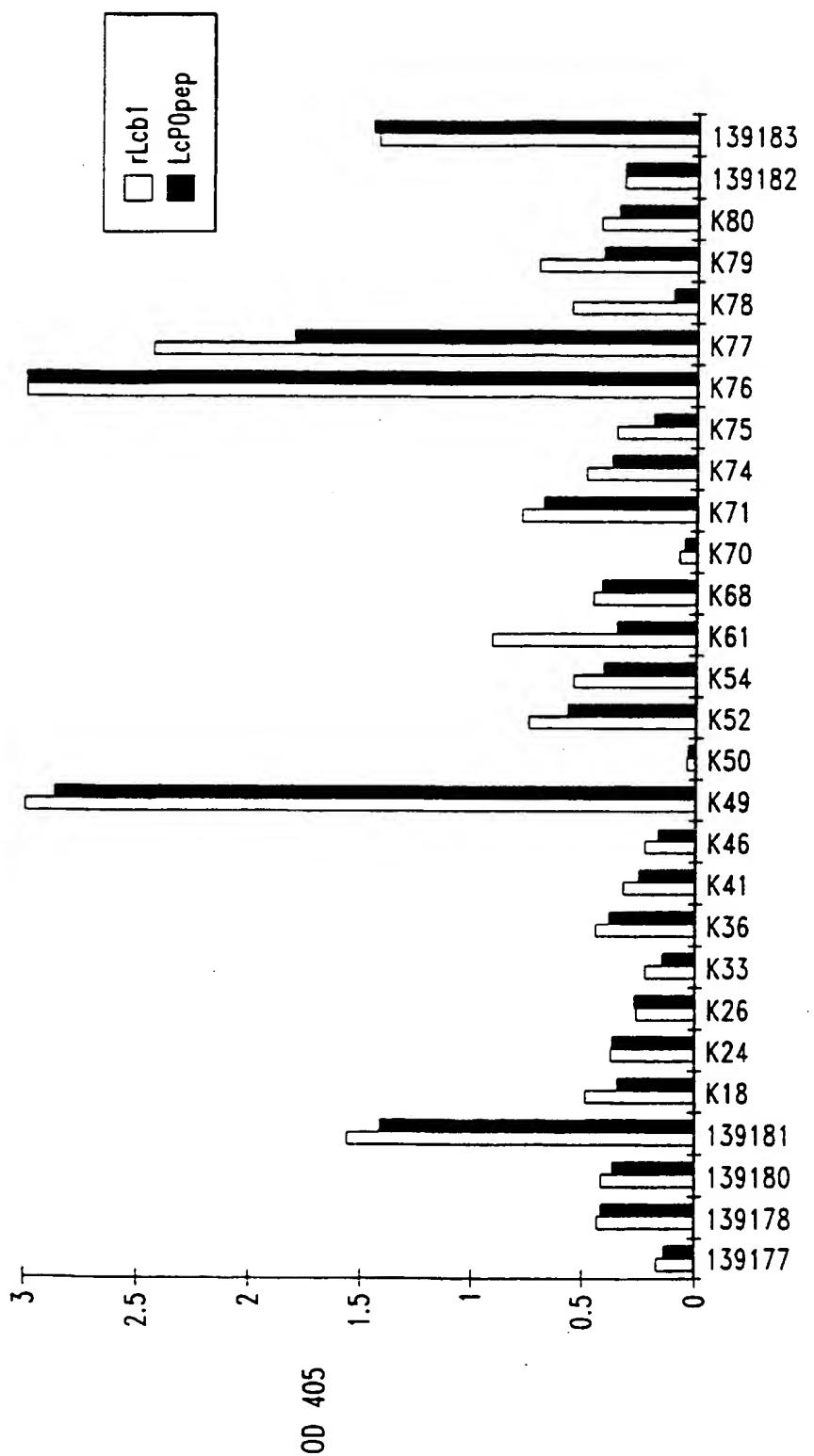


Fig. 5C

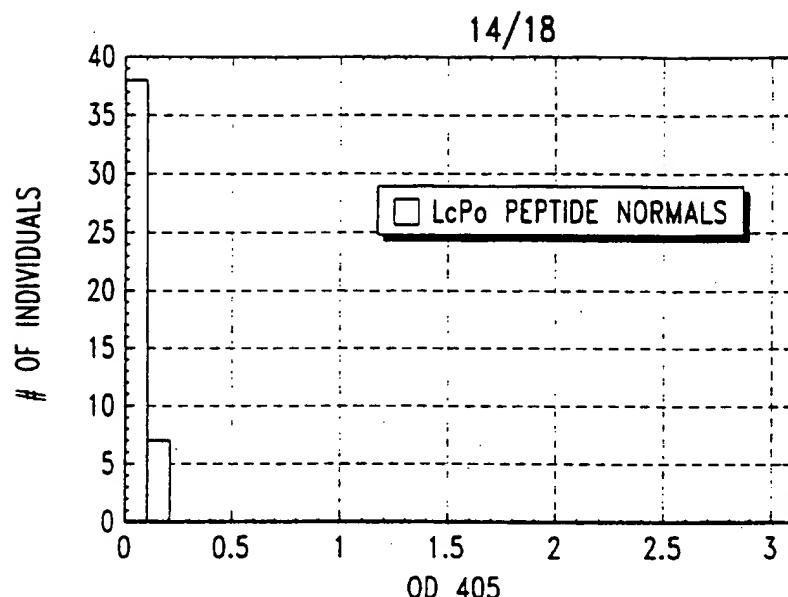


Fig. 6A

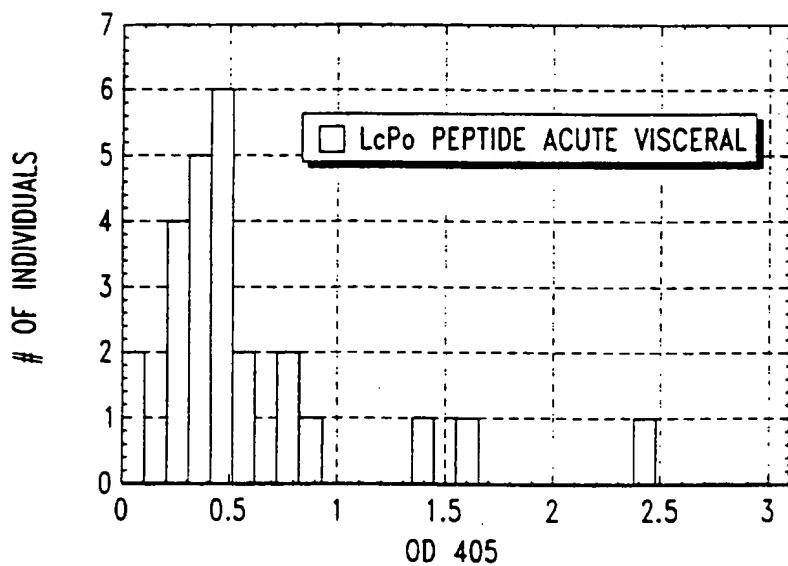


Fig. 6B

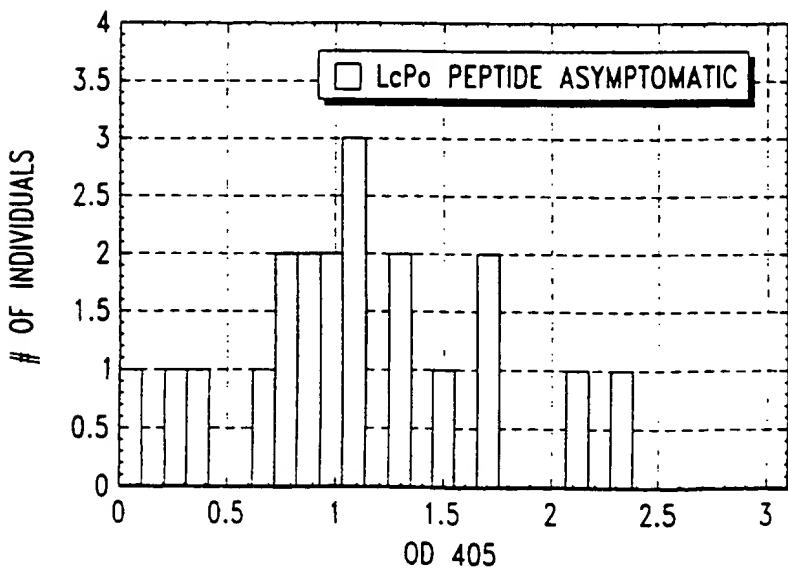


Fig. 6C

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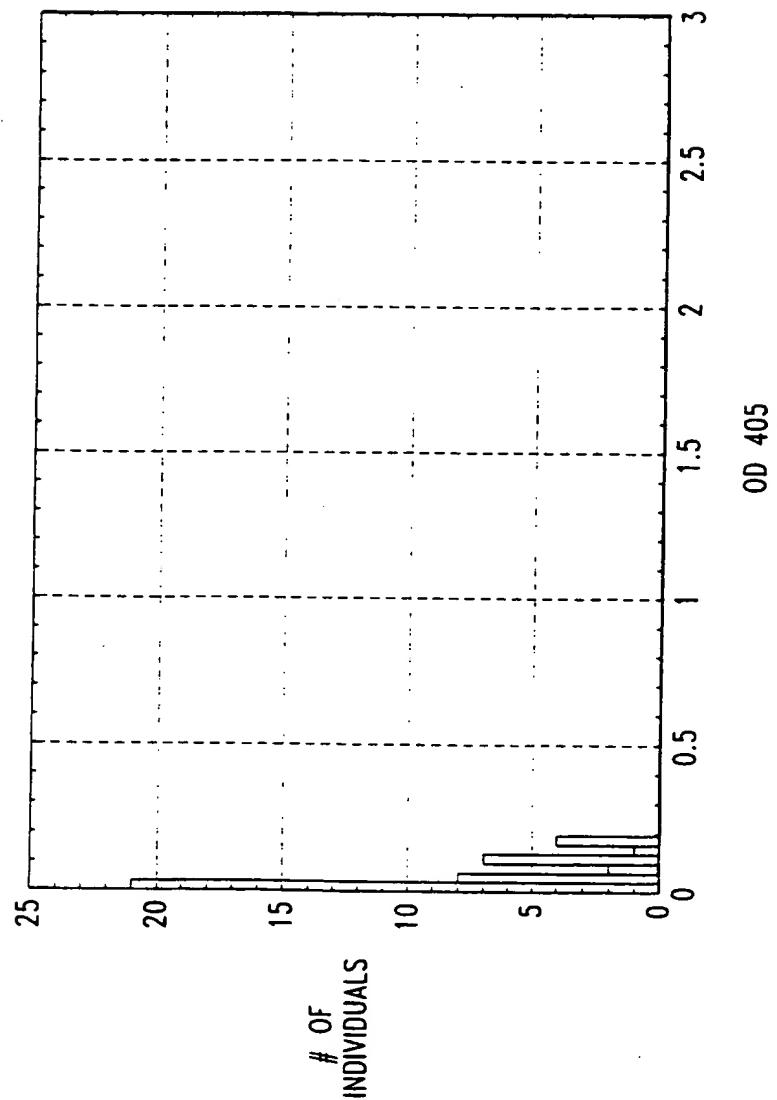


Fig. 7A

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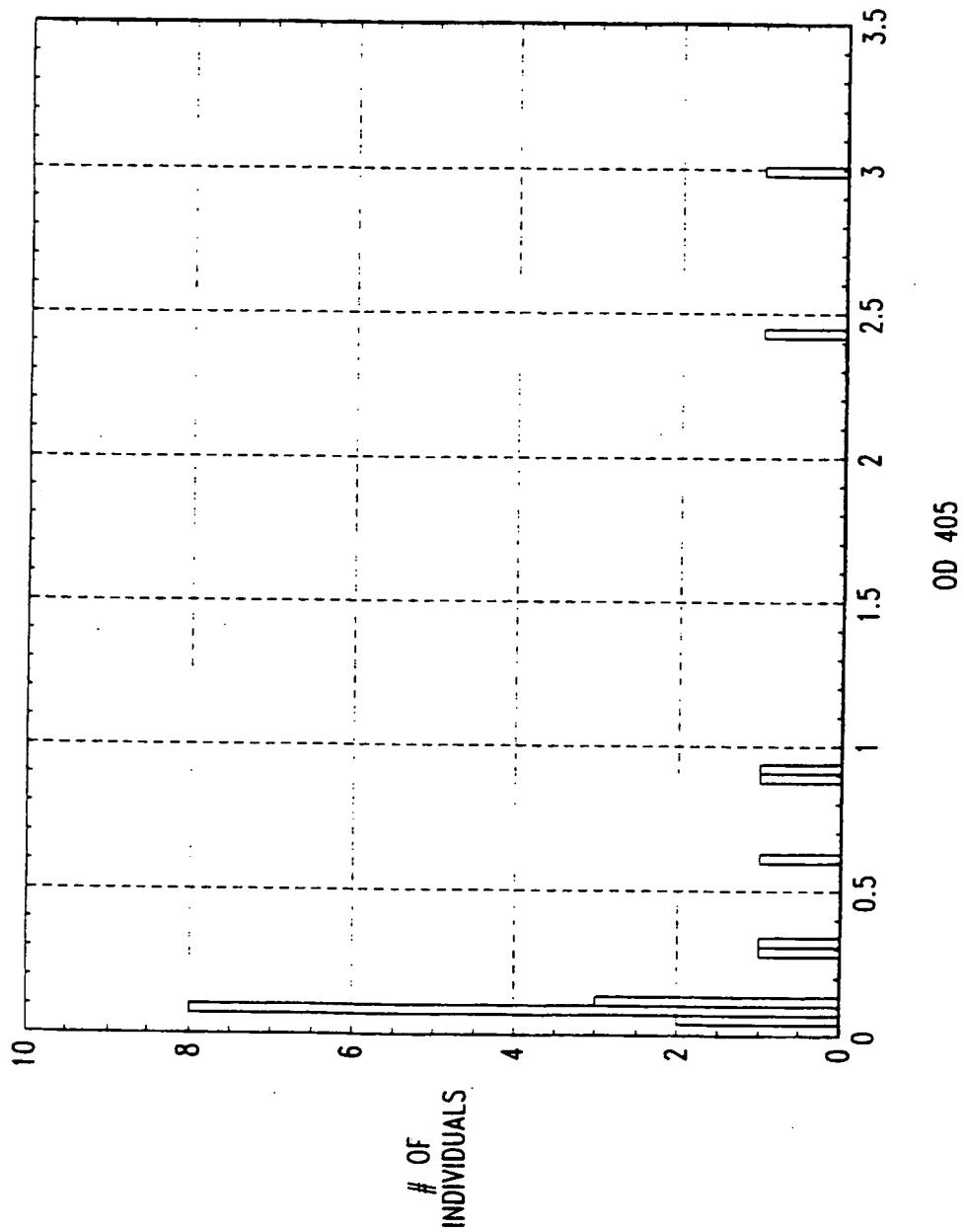


Fig. 7B

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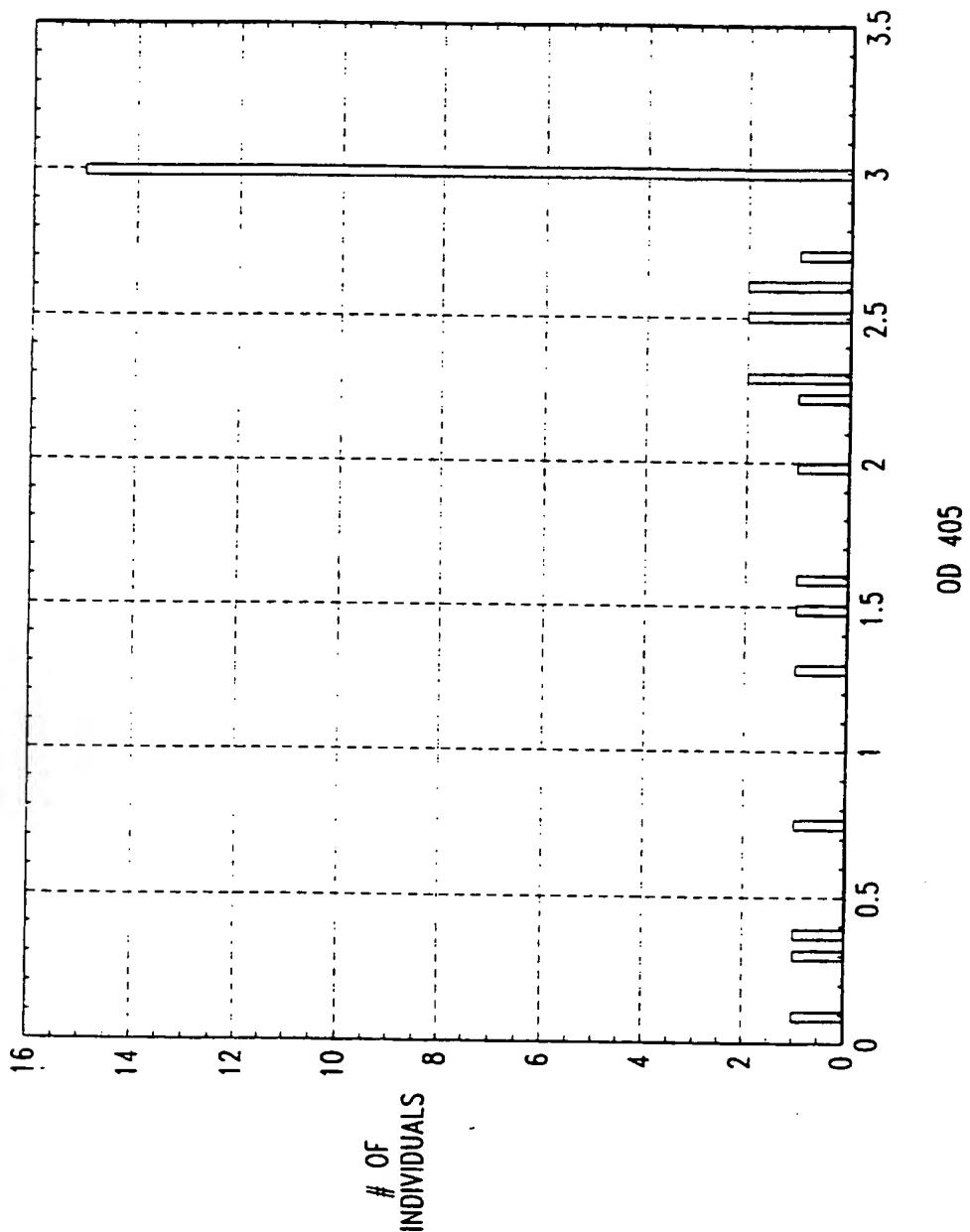


Fig. 7C

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Fig. 8

